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THE RELATION OF THE *GLOSSOPTERIS* FLORA* WITH THE GONDWANA GLACIATION

BY B SAHNI, Sc D , F R S

Professor of Botany, Lucknow University

IN doing me the honour of asking me to preside over the Biological Section, the Council gave the mandate that I should deliver an address on this occasion. As you all know, this annual meeting of the Academy marks the jubilee of its illustrious founder. I should like, at the outset, to ask you all to join with me in congratulating Sir Chandrasekhara Venkata Raman on his completing the 50th year of an unusually distinguished and eventful career.

The relation of the *Glossopteris* flora with the climatic conditions in Gondwana Land has been for many years one of the outstanding problems of palaeozoic botany. Although I have only recently had occasion (Sahni, 1937, pp 217-19, 1938, pp 10ff, 1938 a) to deal with this question I have perhaps a special excuse for speaking on it to-day. Only a few days before I started on my journey to Madras some fresh evidence bearing directly upon the subject was obtained at Lucknow. This evidence is set forth in a paper by Miss C. Virkki which will be read before you this morning (see p 7 below), but I thought it might be of some interest to discuss here briefly the general significance of the discovery of spores in the glacial deposits of Gondwana Land. I may remind you that this discovery was already anticipated by Miss Virkki's earlier work, which was read before the Academy towards the end of 1937, announcing the occurrence of winged pollen grains in the Salt Range in a stratum less than two feet above the Talchur boulder bed (Virkki, 1938).

The idea of a single more or less continuous life-province in the southern hemisphere during the Permo-Carboniferous period arose from the fact that the widely distributed *Glossopteris* flora was so uniform in character: it could only have been evolved under conditions permitting of free intermigration by land plants. Another fact in common between the different parts of the old Gondwana continent is that at a horizon roughly corresponding to the Upper Carboniferous (some think it was in the Lower Permian) there are unmistakable glacial deposits indicating the presence of ground ice. At one

* Address delivered at Madras on December 20, 1938, before the Biological Section of the Indian Academy of Sciences at its Fourth Annual Meeting.

time or another the greater part of this vast area must have been overrun by glaciers and ice-sheets, and in Australia there is evidence of more than one glacial horizon in the Gondwanas, with intercalated plant-bearing beds indicating a relatively warm interglacial epoch. At least some of these Australian tillites are believed to have been deposited in the sea, presumably by floating ice.

It was in a sample of tillite from Bacchus Marsh, Victoria, that the spores in question were found, and some of these spores are indistinguishable from others found associated with *Glossopteris* at several horizons in different parts of India.

Some palaeobotanists, notably Professor Gothan of Berlin (Gothan, 1924, pp 153-55) have held that the Gondwana flora was essentially a flora developed in a warm humid climate like the contemporary flora of the coal measures of Europe and America. Professor Gothan, of course, was not referring to the beginnings of the flora, with which we are here specially concerned, but to the zenith of its development, with its associated coal seams. Probably no one to-day would subscribe to the view that the *Glossopteris* flora originated in a tropical climate. At least in S Africa, remains of this flora have more than once been discovered immediately below the Dwyka tillite (Leslie, 1921, p xxiii, du Toit, 1924, p 75). One of these important specimens, a leaf of *Gangamopteris*, was figured by Professor Seward several years ago (Seward and Walton, 1923, fig 23, Pl XXII). If these occurrences are genuine they can only indicate that the flora was already there when the ice overran the country. The wonder is that many more such occurrences have not been recorded.

As for the interglacial flora in Australia, we have no clear idea how long the interval was and whether it allowed the conditions to become tropical.

I am not aware that any facts bearing upon our problem have been discovered in South America, and our knowledge of climatic conditions in the Permo-Carboniferous of Antarctica is extremely meagre and likely to remain so for many years to come. We may therefore turn our attention to the evidence that we have from the Indian part of Gondwana Land.

A couple of years ago Mr. E. R. Gee kindly sent me some specimens of *Glossopteris*, *Gangamopteris* and other Gondwana plants collected by him in the Salt Range at a horizon only 25 feet above the Talchir boulder bed, and this was the earliest flora then known from the Indian Gondwanas. Some of us, however, have been on the track of still earlier signs of the *Glossopteris* flora. I have long believed that the origin of this flora must have been intimately connected with the glaciation. As I said in 1926, "The close relation of the Gondwana plant beds with the glacial deposits

over enormous areas would alone impose caution against any other interpretation" (Sahni, 1926, p 239) I even gave reasons for the view that some members of the Gondwana flora were probably lineal descendants of the preglacial vegetation. Similar views were expressed nine years later at Amsterdam (1935, p 245) In a paper read before the 1937 Geological Congress at Moscow I suggested that there was "nothing to contradict the view that in its earlier phases the *Glossopteris* flora overlapped with the latter end of the glacial period but this need not mean that it was a glacial flora like the modern flora of the arctic regions " (1937, p 217)

It was therefore an interesting discovery, announced by Miss C Virkki last year, that the spores of some members of this flora had been found in a stratum as low as $1\frac{1}{2}$ feet above the glacial bed. In these early Gondwana shales no leaf impressions have yet been discovered but I think we are fully justified in regarding these spores as the spores of a *Glossopteris* flora, in fact, similar spores were found in *Glossopteris*-bearing shales at higher levels, in the same geological section, including the 25 foot horizon of Mr Gee

These facts made it clear that we were already hot on the track of the pioneers of the *Glossopteris* flora in this part of Gondwana Land. The $1\frac{1}{2}$ foot horizon being practically contemporaneous with the boulder bed, the question was natural "What about the boulder bed itself?" If this flora really existed here in the neighbourhood of the ice, like the modern flora of the Alps and the Himalayas, and that round the edge of the Greenland ice-sheet (Seward, 1922), and, especially, if some members of this flora had winged spores, should we not expect to find at least a few of these spores in the moraines left by the Gondwana ice?

We know that the spores of modern conifers settle down in large quantities year after year on the snow fields and glaciers of neighbouring mountains. From the recent works of Dr V Vareschi (Vareschi, 1935, pp 83-84) we also know that with the peculiar movements of the different parts of the glacier these spores in course of time reach the depths of the glacier, where they mark definite chronological zones even in the folded layers of the ice, like fossils do in the contorted strata of the earth's crust.

If this was what happened to the spores of the early *Glossopteris* flora we should search for their remains in the matrix of the glacial conglomerate, not only in India but also in other parts of Gondwana Land. This is the line of enquiry now being pursued at Lucknow.

A sample of the glacial matrix from the Salt Range was examined but gave a negative result. Another sample, from the Dwyka tillite in South Africa, also yielded no spores. But a fragment of the glacial matrix from

the well-known tillite of Bacchus Marsh in Victoria (Australia) revealed not only a number of different kinds of spores, both winged and unwinged, but also some shreds of pitted tracheids. And the interesting point is that at least some of the winged spores closely resemble others found in the Lower Gondwana shales of India. It would be premature, if not rash, to assert that the glacial spores from Bacchus Marsh are specifically identical with the Indian spores. But at least it is safe to say that, like the latter, they are the spores of some early members of the *Glossopteris* flora. Perhaps some day we shall get to know what the parent plants of these spores were. But this at least we may now say with confidence, that the *Glossopteris* flora actually co-existed with the Gondwana ice in Australia, as it did in South Africa and almost certainly in the Salt Range. The chances are that when all the samples, now being received for investigation, thanks to the kindness of geological colleagues in various parts of the world, have been examined, the evidence will accumulate in favour of the view that this flora originated during the Ice Age, possibly by a process of differentiation among the few survivors of the pre-glacial vegetation. Exactly how this phenomenal evolution of new forms took place, and whether the cold had directly to do with it, as I have suggested elsewhere (1938, p 13, 1938a) is another matter.

A few words may be added concerning the localities and horizons from which the Indian spores, comparable with those from Bacchus Marsh, were obtained. They occur not only in the Salt Range at horizons 1½ feet, 4½ feet and 25 feet above the Talchir boulder bed, but also in a *Glossopteris*-bearing shale referred to the Barakar horizon (Lower Permian) in the Daltongunj Coalfield (Behar) and in *Glossopteris*-bearing shales from Rewah in Central India, both of which lie far above the glacial horizon. If, as presumed, all these spores belonged to the *Glossopteris* flora, it would appear that this flora became acclimatised to the relatively warmer (though still temperate) conditions which are indicated by its great profusion in the higher strata, which also contain thick seams of coal (see Sahni, 1937, Table p 218). Nor is the Talchir flora quite the same as the flora of the higher beds.

I ought to add that the spores I have so far mentioned are chiefly of the type with a single circular wing, attached like a frill round the body. In addition to these there occur two-winged spores of the *Pityosporites* type, recalling the solitary spore *P. antarcticus* figured by Professor Seward from the Beacon sandstone in Antarctica (Seward, 1914, 1933a). Spores of this latter kind were discovered by Miss Virkki towards the end of 1937 and these were also found at the 1½ ft and 25 ft horizons already mentioned in the Salt Range, in the *Glossopteris*-bearing shales in Rewah (Central India); and in a piece of shale from the Newcastle Series in New South Wales which was

crowded with incrustations of *Glossopteris browniana*. Similar winged spores have also been reported from South Africa (Seward, 1933 a). The occurrence of these spores in areas far and wide in Gondwana Land is fully in accord with the wide distribution of the *Glossopteris* flora.

While we may now feel satisfied that a definite time relation has been established between the advent of the *Glossopteris* flora and the Gondwana glaciation, many details of the picture still remain to be filled in. This will no doubt be done as we progress with the microscopic investigation of the early Gondwana deposits, including the tillites. But a further and important step in connection with the same enquiry would be an investigation of the microflora of the strata immediately preceding the glaciation, of which at present we have no information whatever.

This recent work, necessarily of the nature of a reconnaissance, serves to focus attention on the importance of the study of microfloras as an aid to geology. Geologists in this country had always told us that the lowermost strata of the Talchur series were unfossiliferous. Until recent years, no one had seriously questioned this statement, for ordinarily one does not expect signs of life in a wilderness of glaciers and ice-sheets. But it was not the boulder bed alone that was said to be devoid of fossils. Dr. C. S. Fox of the Geological Survey of India, to whom we owe a most detailed and valuable account of "The Gondwana System and Relation Formations", wrote not long ago that the *Glossopteris* flora did not come into evidence till the highest beds of the Talchur stage were being laid down (Fox, 1931, pp. 8, 13, 14, 1934, p. 12, 1937, pp. 216-17). He believes that the climate of the Ice Age was unsuited to the *Glossopteris* flora.

It is not always an easy task for the student of modern plants to assess the climatic relations of an unfamiliar flora placed before him in the form of herbarium sheets. To attempt such a problem with an extinct flora known almost solely from leaf impressions must be a much more difficult task. We are, of course, still only at the threshold of the problem. But those who have long been familiar with the *Glossopteris* flora and have given patient thought to the several aspects it presents, both in the field and under the microscope, agree that it probably represented the vegetation of a cold temperate climate, at least as far as its earlier history is concerned (Seward, 1933, p. 258).

Dr. Fox also, of course, regards it as a temperate flora, thereby striking the comfortable mean between the torrid and the frigid, which we all like. But when we find the remains of the flora not only closely associated with, but actually within, the glacial boulder bed, the facts do seem to weigh rather on the cold side.

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ON THE OCCURRENCE OF SIMILAR SPORES IN A LOWER GONDWANA GLACIAL TILLITE FROM AUSTRALIA AND IN LOWER GONDWANA SHALES IN INDIA

BY C VIRKKI, B A , M Sc

Research Student, Department of Botany, University of Lucknow
(With one plate and six figures in the text)

Received January 5, 1939

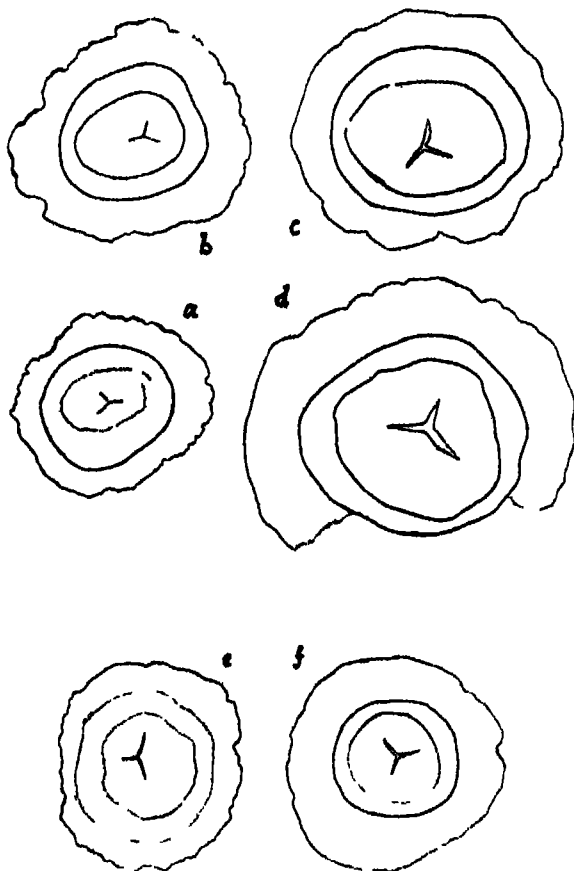
(Communicated by Professor B Sahni, F R S)

IN a previous communication (Virkki, 1937) I described some double-winged spores of the type *Pityosporites* Seward obtained from the Salt Range, from a horizon as low as 1½ ft above the Talchir boulder bed—the lowest plant-bearing horizon so far recorded from the Gondwanas of India. Spores of the same type were also found in shales at higher horizons, where they were associated with *Glossopteris*, etc., both in India and Australia. The occurrence of spores in these early Gondwana shales gave room for hopeful speculations that spores might be obtained even from the glacial matrix itself. In a recent address at Calcutta Professor Sahni (1938, p 16) said "To prove that the flora actually co-existed with the ice it only remains to demonstrate these spores within the glacial matrix itself, and I think the facts at hand fully justify the expectation that this will be done." Accordingly at his suggestion the investigation of the glacial matrix from the Permo-Carboniferous of the various remnants of Gondwana Land was taken up. In one of these tillites from the well-known glacial horizon at Bacchus Marsh, Victoria, spores were found in considerable variety and number. Some of these are figured in this preliminary note. Although most of them are not of the *Pityosporites* type they closely resemble others found associated with *Pityosporites* in the Salt Range shales.

A description of all the spores obtained will be given in a detailed paper to be published later. For the present a brief note on the significance of the occurrence of these spores in the glacial matrix of Gondwana Land will suffice.

The authorities of the British Museum kindly sent fragments of Gondwana tillites from Cape Colony and from Bacchus Marsh in Australia. The hardened Dwyka tillite from South Africa was treated with dilute hydrofluoric acid, washed in water and examined for spores, but it gave a negative

result. Schultz's maceration fluid (nitric acid and potassium chlorate) was used for the less hardened matrix from Australia and it yielded several kinds of spores. A fragment about .05 c.c. in volume yielded as many as five different kinds of spores and a few bits of tracheids. The total number of spores obtained was at least twenty-five and this number does not include those lost in manipulation.



TEXT-FIGS a-f Camera lucida sketches of some of the spores in Plate I, drawn to show their triradiate alveoli. See explanation of Plate. All $\times$ ca. 360.

Among the spores obtained those with one circular wing all round seem to be very common. They occur in different sizes. Some of the spores show a close resemblance with others obtained (but not yet figured) from shales at different Lower Gondwana horizons in India. In the present note only the one-winged spores from Australia and from India are figured. The body, with a comparatively thick rim, is more round than elliptical in the flattened

condition. In well-preserved spores a triradiate slit is clearly seen. The frill-like wing is radially striated.

A sample of the glacial matrix collected by Mr K. Jacob and myself last year from the Salt Range did not yield any spores, but some further specimens from the same region kindly sent by the Director of the Geological Survey of India still await examination. However, spores very similar to the Australian ones were obtained (a) from shales 1½ ft, 4½ ft and 25 ft respectively above the Talchir boulder bed in the Salt Range, (b) from shales intercalated in the Middle Productus Limestone of the same region, (c) from certain black *Glossopteris*-bearing shales from Palamau in the Daltongunj coalfield, Behar, and (d) from black *Glossopteris*-bearing shales in the South Rewa Gondwana basin, Central India. Of these spores from the Indian Gondwanas a few of those which resemble the Australian ones are figured here for comparison (Pl I, Figs 6-11, Text-Figs e, f).

On a comparison of the spores from Australia and India one is impressed by their remarkable resemblance with one another in their fundamental features, such as the more or less round body with a thick rim, the triradiate slit and the radially striated, frill-like wing. The spores are provisionally divided into five groups according to their size (Pl I, Figs 1-5). The measurements of the whole spore including the wing, as well as those of the body alone, are given separately in the explanation of the plate. There are slight differences between the spores within each group itself, but these may be due to the irregularity of the margin and to the mode of preservation of the spores.

It is impossible to determine the probable affinities of these isolated spores. With the meagre data at hand we are not in a position to draw any definite conclusion. Yet, it might not be unreasonable to think that the plants represented by these Indian and Australian spores, which resemble each other in their fundamental features, were closely related if not identical, for it is often observed in the case of living plants that spores belonging to members of one group show certain features characteristic of it. Further, from the study of impressions, especially of fronds, we are already in a position to say that similar as well as identical types of plants existed in distant parts of Gondwana Land.

The discovery of the spores in the glacial matrix supports the view of Professor Seward (1929, p. 12), Professor Sahní (1938, pp 11-16), Dr du Toit (1924, p 75), Jongmans and others that the *Glossopteris* flora might have co-existed with the Permo-Carboniferous Ice Age. In Africa, as early as 1921, Leslie (1921, p 23) recorded the occurrence of fairly well-preserved impressions of *Gangamopteris* immediately below the

Dwyka tillite Dr du Toit (1924, p. 75) made a similar discovery in another locality in South Africa At Bacchus Marsh, in Australia, according to Süssmilch (1922, p 144) *Gangamopteris* and *Schizoneura* were found in beds interstratified with the glacials In the present paper spores are described from a tillite at the same place and similar spores have been found in India in shales 1½ ft, 4½ ft, and 25 ft above the Talchur boulder bed in the Salt Range On the occurrence of the spores in these shales, Professor Sahní (1938, p 16) writes " the time interval represented by the overlying 1½ ft of sediments must have been so short that the lowermost of the spore-bearing horizons may be taken to be approximately of the same geological age as the glacial bed ".

The above mentioned records of the occurrence of leaf impressions closely associated with the glacial deposits in widely scattered areas of Gondwana Land, and of the spores in the glacial matrix itself, make it difficult to accept Dr Fox's conclusion (1931, p 14) " that the climate of the Ice Age was not favourable to the *Glossopteris* flora " In support of his view he draws our attention to some " important factors ", and adds, " There is no doubt that a *Glossopteris* flora has *not* been found closely associated with the glacial boulder beds of the Gondwana system In Kashmir and the Punjab Salt Range the *Gangamopteris* (with *Glossopteris*) horizon occurs at a considerably higher horizon than the boulder bed " Again in 1937 (Fox, 1937, pp 216-17) he wrote " The *Gangamopteris* flora does not appear until the highest beds of the Talchur stage were laid down, and, judging by specimens from Rikba, *Gangamopteris* had stiff, thick, aloe-like leaves, quite unlike plants of a cold area. "

On the other hand evidences are accumulating in favour of the view that the *Glossopteris* flora originated during the cold age, although even so it need not be regarded as a glacial flora We are still in the dark as to the origin of this flora indigenous to the Southern Hemisphere According to Professor Sahní (1938, pp 13-14) it is not impossible that the *Glossopteris* had their ancestors in the pre-glacial period The poor flora which survived the glaciation must have given rise to the rich flora of Gondwana Land Anyhow we can now say with certainty that the *Glossopteris* flora existed at least in the Permo-Carboniferous Ice Age, if not earlier

Another importance of the spores from the tillite of Australia is their striking resemblance to the Indian spores from South Rewa and Behar which occur in shales far above the glacial horizon This shows that the plants to which these spores belonged survived the Ice Age and persisted till long after the Gondwana glaciation

Occurrence of Similar Spores in a Lower Gondwana Glacial Tillite 11

I take this opportunity to express my deep gratitude to Professor B. Sahni, F.R.S., for his ready suggestions and invaluable guidance. I am indebted to the authorities of the British Museum for kindly sending us the tillites of the Permo-Carboniferous of Australia and Africa. This work has been carried out during the tenure of a Research Fellowship for which I have to thank the University of Lucknow.

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EXPLANATION OF PLATE

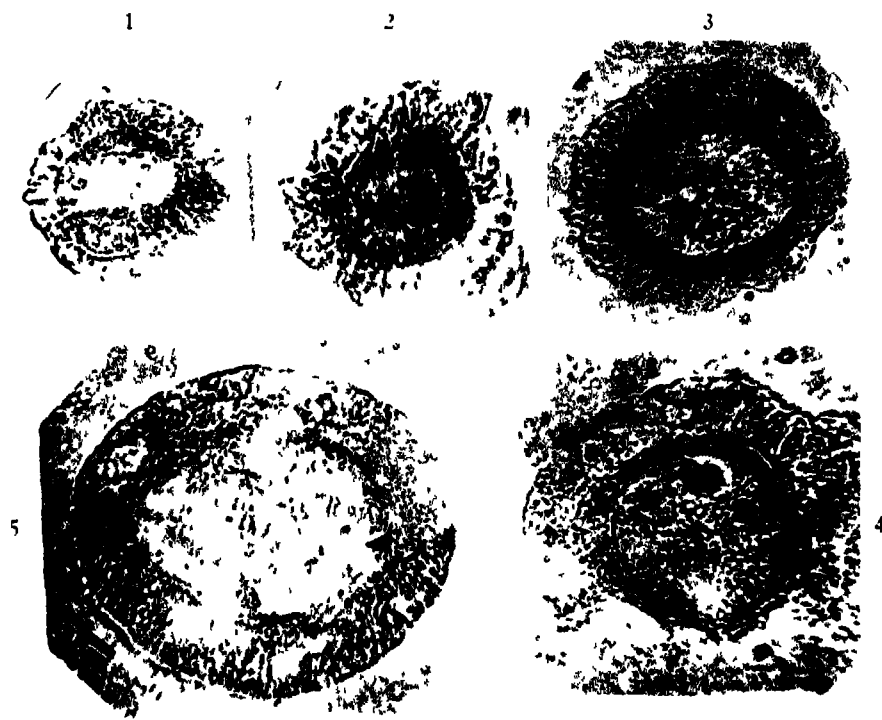
The photographs are all from untouched negatives. The original slides are preserved in the Department of Botany, Lucknow University.

All the spores are magnified $\times 380$. For the actual sizes see table below.

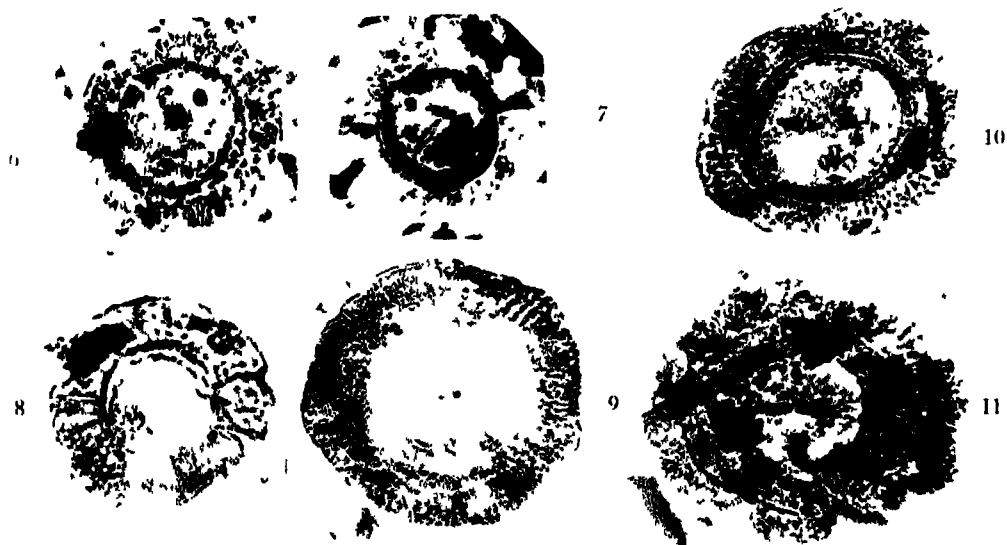
- Figs. 1-5** From a glacial tillite at Bacchus Marsh, Victoria (Australia). Spores 1-4 are also sketched in Text-Figs. a-d respectively to show their triradiate slits.
- Figs. 6-9** From shales at low horizons in the Salt Range (India). 6 is from a level $1\frac{1}{2}$ ft., 7 from $4\frac{1}{2}$ ft. and 8-9 from 25 ft. above the Talchur boulder bed. The shales at 25 ft. contain a well-developed flora including *Glossopteris*, *Gangamopteris*, *Samaropsis*, etc. The spore 6 is also seen in Text-Fig. e, and spore 8 in Text-Fig. f.
- Figs. 10-11.** From a Lower Barakar shale, bearing a *Glossopteris* leaf, at Palamau in the Daltongunj coalfield, Behar (India).

DIMENSIONS IN μ

1	Complete spore	74 × 63 ;	Body	49 × 45.
2.	„	86 × 72 ;	„	53 × 47
3	„	96 × 87 ,	„	72 × 63
4	„	118 × 109 ,	„	83 × 68
5	„	150 × 114 ,	„	108 × 72
6	„	75 × 75 ;	„	50 × 49
7	„	67 × 67 ,	„	50 × 45
8.	„	80 × 74 ;	„	46 × 43
9	„	100 × 100 ,	„	76 × 76
10	„	93 × 86 ,	„	64 × 64
11.	„	114 × 100 ;	„	79 × 70



1-5 Bacchus Marsh Follite 380



6-9 Salt Range
Lower Gondwana Shales
380

10-11 Daltongunj
Coalfield, Behar
Lower Gondwana Shales
380

STUDIES IN THE DISEASES OF *MANGIFERA INDICA* LINN.

I. Preliminary Observations on the Necrosis of the Mango Fruit with Special Reference to the External Symptoms of the Disease

BY S N DAS GUPTA AND G S VERMA
(From the Department of Botany, Lucknow University)

Received May 30, 1938

Introduction

THE mango (*Mangifera indica* Linn, Fam Anacardiaceæ) is an important fruit tree of India which is cultivated in most of the provinces of the country. Until recently very little interest was taken either by the Government or by the growers to raise improved varieties or to improve the mango cultivation. In recent years thanks to the enterprise of the Imperial Council of Agricultural Research and of the agriculture departments, the mango cultivation is receiving a very strong impetus particularly in Bihar and in the United Provinces. Considering the importance of the fruit, however, the investigations carried out in India on the diseases of mango are extremely meagre. In this and subsequent papers of this series it is intended to deal with the diseases to which the mango tree and the mango fruit are susceptible.

This paper which is the first of the series deals exclusively with our preliminary observations on the necrosis of the mango fruit with particular reference to the external symptoms of the disease. The work is only in its initial phase but the authors venture to publish the results with the hope that the disease will attract the attention of other scientific workers who might be in a position to collect information regarding the disease in their respective provinces and localities. Co-operation of scientists all over India is required to get a correct estimate of the incidence and extent of the disease and the financial loss involved.

The necrosis of the mango fruit is definitely known in two of the most important mango-growing provinces of India, namely, Bihar and the United Provinces. It most probably occurs in Bombay and Bengal, and we venture to think that proper investigation will prove the existence of the disease in other mango-growing provinces as well.

The occurrence of the necrosis must have been observed long before it was actually recorded. So far as the writers are aware the first record of

the disease is from Bihar, it was made in the year 1908 by Woodhouse* (1909), the then Economic Botanist to the Bihar Government. He writes, "Round Bhagalpur mangoes do not seem to be much affected by disease, but this year much fruit is said to have been spoilt at the Sabour Farm by the smoke from the brick-kilns which apparently *blacken the apex of the mango and interferes with its development*" (The italics are ours). The disease has also been referred to by Naik (1934) in a Hindi agricultural journal, by Allan (1936) and by Pal and his colleagues (1937).

In the United Provinces the disease was first observed in the year 1923 when reference was made to Mr P K Dey, the Plant Pathologist to the U P Government. Since then the disease is apparently increasing at a tremendous rate, as judged from the enquiries and complaints received from orchard owners every year.

The occurrence of the necrosis of the mango fruit and the extent of financial loss caused by the disease can be ascertained by means of an intensive and extensive survey of the mango-growing areas in different provinces by men thoroughly acquainted with the symptoms of the disease. It is a task that can be carried out only with the active co-operation of the agricultural departments and botanical institutions of different provinces. No such attempt has yet been made in any province even where the disease is quite prevalent.

In the United Provinces the disease goes by the popular name of *Koeli*. But the term includes two different diseases altogether. One is the necrosis in which the blackening and the disintegration of the tissue invariably starts from the apex of the fruit, and from nowhere else, long before the mango is ripe, the other in which the dark spots appear in any part of the skin of the fruit, including the apex. The two diseases are entirely different in character, still they are confused by the villagers from whom information regarding the prevalence of the disease in preceding years, and the first incidence of the disease in the locality, are usually taken. While surveying, therefore, it is necessary to exercise the utmost caution in accepting the opinions of the villagers. They are better left out of consideration altogether unless their descriptions are substantiated by facts.

The authors carried out a detailed survey of the disease in a number of orchards in the neighbourhood of Lucknow situated on Kursi Road, Barabanki Road, Sitapur Road, Malihabad Road and Malihabad proper during the mango season of 1937. The number of orchards kept under

* The authors' thanks are due to Dr. T. C. N. Singh, D.Sc. (Luck.), Assistant Economic Botanist to the Bihar Government, for the reference.

observation was twelve, and of these three were utilised for special work regarding the necrosis. All these orchards contained the most superior qualities of mangoes that are grown in the United Provinces. For example, Dasehri, Safeda, Gola-Mohanbhog, Fazli and others. All these are "graft" mangoes. In the Kapurthala garden, however, there are a number of *Tukhmi*† mango trees which were also included in our observation. These orchards are well kept and well cared for. Although as many as twelve orchards were kept under observation the number is insignificant in comparison with the total number of orchards in Lucknow and other important mango-growing centres in the United Provinces, namely, Unao, Cawnpore, Hardoi, Farrukhabad, Bareilly, Rampur, Moradabad, Saharanpur, Dehra Dun, Bulandshahar, Gorakhpur and Benares, etc. Nevertheless the results obtained from the survey cannot be ignored and the further information that we expect to get in the mango season of 1938 will supplement and not supplant the result obtained last year.

The following are the three orchards kept under special observation

TABLE I
Orchards kept under Special Observation

Name by which orchard is referred in the text	Location	Owner
1. Kapurthala	2nd mile, Kursi Road, Aliganj, Lucknow	Kapurthala State
2. Bahadurpur	Off 4½ miles Kursi Road, Village Bahadurpur, Lucknow	Sheikh Abdur Rahman
3. Zahurpur	5 miles Kursi Road, Village Zahurpur, Lucknow	Mr. Devi Shanker Shukla

The Bahadurpur orchard was utilised for most intensive work and was normally visited twice every week and more often when necessity demanded.

Some difficulty was experienced with regard to the identification of the variety of mango in the orchard. Owing to the fact that in some cases no proper record was available reliance had to be placed on the names given by the gardener in charge of the orchard.

† *Tukhmi* [*Tukhm*=Seed (Persian)]. Growing from seed as opposed to graft. Fruit mostly fibrous.

Damage—An attempt was made to ascertain the total percentage of mango crop damaged annually by necrosis in all the mango-growing provinces in India and in the United Provinces in particular by inviting figures from the agricultural and marketing departments. But no figures were available. This is due to the fact that there has been no organised survey in the affected provinces to collect information on the point.

The following few facts relate to the damage done in the orchards kept under our observation. It was seen that the necrosis occurred in all the orchards excepting those in Malhabad proper. The extent of damage in the affected orchards varied. Of the three special orchards mentioned the damage was most heavy in the Bahadurpur orchard where the entire crop was heavily infected. Dasehri suffered most, the damage being 100 per cent. The other varieties showed relatively less infection. The crop in the Kapurthala garden too, was heavily infected. Here again Dasehri showed cent per cent infection. Two varieties in this garden proved to be resistant. The damage was least in the Zahirpur orchard. It will be apparent from the above that the extent of damage in an orchard depends on the varieties grown. Orchards having the resistant varieties suffer the least. But the best varieties of mangoes are most susceptible. The question of varietal resistance is discussed elsewhere.

Financial Loss—An attempt was also made to ascertain the financial loss due to the necrosis in the U P or elsewhere in India. But as in the case of the damage no figures were available. The authors had naturally to take resort to the reported financial loss sustained by the owners of the orchards under observation. The financial loss, it should be remembered, does not depend solely upon the amount of crop damaged but is dependent upon various other factors, namely, the total production, demand, etc., of the mango for the year under consideration.

The following figures as obtained from the orchard owners give only an approximate idea of the financial loss sustained by them.

Before the incidence of the disease the mango crop of Zahirpur orchard used to be sold for Rs 700–Rs 1,000 per year according to the amount of yield. Since the advent of the disease there was a marked drop in the income. The condition grew so bad that the orchard was abandoned. The condition of the orchard has improved lately. The disease is comparatively less severe and the crop last year was sold for Rs 500.

The mango crop of Bahadurpur orchard used to be worth about Rs. 800–Rs 1,000. Since the incidence of the disease the income has substantially decreased. Last year (1937) the crop was sold for Rs. 113 only. The crop

of the Kapurthala garden was sold for only Rs 50 last year (1937) Its yield was relatively less than in other gardens

Symptoms of the Necrosis

Altogether twenty-two varieties of mangoes, fifteen grafts and seven *Tukhm*, from the Bahadurpur orchard and the Kapurthala orchard were kept under close watch to keep a complete record of the external symptoms of the disease from its very inception to the final stage The symptoms are more or less the same for all the varieties that are affected with necrosis The differences that exist are differences of degree only rather than anything of fundamental nature The following is a description of the symptoms of the disease as found in Dasehri mangoes (Plate II, Figs 2-12)

The first external symptom that heralds the incidence of the necrosis is the appearance of a small ætiolated area at the distal end of the mango fruit against the general green colour of the skin The ætiolated area gradually increases in size and intensity and covers the entire tip Generally, however, before complete ætiolation has taken place isolated greyish spots of indefinite outline make their appearance. The tip at that period assumes a dirty green colour—a mixture of yellow and green As the disease progresses the spots become dark-brown, increase in size and finally coalesce to form a continuous necrotic area This is the advanced stage of necrosis Due to the decay of the tissue the necrotic portion collapses At this stage the diseased mango fruit shows three distinct regions externally (Plate II, Figs 6-9)

- 1 The healthy green part which constitutes the major portion of the fruit, towards the stalk end
- 2 The necrotic part, a much smaller area than the former, at the distal end.
3. A narrow zone between the two major areas, which consists of the ætiolated skin

The next stage in the development of the disease is complete disintegration of the pericarp and the mesocarp of the necrotic area exposing the flesh which shows a dark-brown colour As more and more of the pericarp and mesocarp tissues disintegrate, the stone of the fruit emerges and remains protruding beyond the flesh The protruding stone has a layer of collapsed tissue over it, the thickness of the layer depending upon the degree of disintegration the mesocarp tissue has undergone The sequence of symptoms will be at once apparent from Plate II, Figs 2-12

The symptoms described above for the Dasehri variety are also shown by the Safeda and certain *Tukhm* varieties. In the Gola-Mohanbhog, however, where the attack is less severe, the disease does not proceed beyond

the earlier stage (Plate II, Figs 14-19) In this variety etiolation first makes its appearance at the distal end and is followed by brown spots which coalesce There is no further advance of necrosis, and the disintegration of the tissue and the consequent emergence of the stone is not known to occur

So far as our observations go the necrosis in Dasehri usually extends up to a sixth of the length of the fruit. In Safeda and the *Tukhm* varieties usually a quarter of the fruit becomes necrotic In other varieties such as Gola-Mohanbhog, etc., the disease is restricted to a very small area at the apex of the fruit The authors have not yet come across any fruit of any variety where the necrosis claims the whole of the mango This is likely to occur in specially favourable condition to the disease The extent of damage in a given fruit must be directly proportional to the intensity of the disease Although the whole mango may not become necrotic the apparently healthy portion—the portion which is not directly affected, loses the taste and becomes unfit for human consumption

In many cases the disease is accompanied by *Gummosis*, that is, the exudation of drops of gums from the necrotic region The amount of exudation varies not only with the varieties but also within a given variety. *Gummosis* is most copious in the Gola-Mohanbhog variety

Varietal Resistance to the Necrosis

In the Bahadurpur orchard and in the Kapurthala orchard altogether twenty-two varieties of mangoes, fifteen graft and seven *Tukhm*, were kept under observation It was found that all the varieties were not affected to the same extent as judged from the percentage of fruit damaged. In the following table the graft varieties are arranged in order of their susceptibility as found for the Bahadurpur orchard It will be seen that in certain cases the same variety has been entered separately in Table II This is done only in those cases where the apparently same variety growing in different orchards shows great difference in the extent of damage

It will be seen from Table II that the Dasehri variety is most susceptible to the disease (100 per cent). The fact has been repeatedly confirmed from our inspection of other orchards If any variety is likely to be affected in a given orchard it is Dasehri. Of the other varieties in the Bahadurpur orchard Gola-Mohanbhog, Fazli and Langara come next in order, with an estimated loss of 90 per cent Khajuri shows a loss of 75 per cent. and Amin, Safeda (Lucknow) and a certain Bombay variety about 50 per cent. Loss in the Malda variety is only 10 per cent. There is no completely immune variety in this orchard.

TABLE II

Susceptibility of the Graft Varieties to Necrosis Arranged in order of their susceptibility as found in Bahadurpur Orchard

Variety	Loss in percentage (approximate)	
	Bahadurpur orchard	Kapurthala orchard
Dasehri .	100	100
Langara ..	90	75
Fazli . .	90	.
Fazli		50
Gola-Mohanbhog .	90	.
Khajuri . .	75	..
Khajuri (Bombay) ..	.	75
Khajuri .	..	10
Amin . .	50	.
Sundari ..	50	
Bombay .	50	..
Bombay . ..	..	10
Safeda (Lucknow) ..	50	..
Safeda (Maliabad) ..	..	10
Safeda (Narma) ..	..	nil
Malda .. .	10	..
Kesari . .	..	20
Kalapahar	..	20
Tamburia ..	..	nil

The results obtained in the Kapurthala garden are somewhat different. The amount of damage on the whole is slightly less. The order in which the different varieties would be arranged according to the incidence of disease is also slightly different. There are two completely immune varieties, namely, Safeda (Narma) and Tamburia.

One more interesting point to be remembered in this connection is that not only is there a varietal difference in the resistance, but there is a sub-varietal difference as well. For example, in the Safeda different sub-varieties are susceptible to different degrees. Thus the Safeda (Lucknow) shows about 50 per cent loss due to the disease, the Safeda (Mauhabad) shows 10 per cent loss while the Safeda (Narma) is completely resistant. Similarly among the three sub-varieties of the Khajuri, the loss is 75 per cent in two sub-varieties and 10 per cent in another.

The difference might be due to the fact that the conditions in the two orchards are not strictly comparable. Hence the same variety shows different degrees of damage in the Bahadurpur and Kapurthala orchards. It is not improbable, however, that different varieties are passing under the same varietal name. Or perhaps, there is a real difference in the susceptibility within the variety or again it might be an example of individual resistance.

There is a varietal difference in the resistance of the *Tukhmi* mangoes as will be evident from Table III.

TABLE III
The Tukhmi Varieties arranged in order of their Susceptibility to the Necrosis (Kapurthala Orchard)

*Name of Variety	Per cent. loss (a rough estimate)
Pantahia ..	75
Lambauni .	75
Baheli ..	..
Sahtnia ..	..
Basaindha .	..
Paharia ..	..

* The names were obtained from the gardener. Their correctness therefore cannot be vouchsafed.

It will be seen from Table III that among the *Tukhmi* mangoes Pautahia and Lambauri are susceptible and four other varieties are almost completely free from the disease

An interesting fact was observed in this connection. The *Tukhmi* trees in the Kapurthala garden grow in pairs and the two members of each pair belong to a different variety, viz, Pautahia variety grows with Basaindha variety and Lambauri variety grows with Baheli variety. The first mentioned member of the pair is in each case susceptible. It is a conclusive proof of the varietal resistance to the necrosis, since in both the cases the members are growing in the identical edaphic and climatic conditions

Probable Cause of the Disease

1 *Parasitic Organism*—As the necrosis may well be caused by some parasitic organism like bacteria and fungi, attempts were made to isolate any organism that might be responsible for the disease. For this purpose portions of diseased tissue were taken and put into slants of various nutritive media and were kept at room temperature. Periodic isolations were attempted from diseased tissues of fruits of different varieties showing different degrees of rotting. More than 50 cultures were attempted each time but always with the identical result that no organism appeared in the culture. The disease does not seem to be due to a fungus or to a bacterium.

2 *Virus*—Attempts were made to ascertain if the disease was due to virus by inoculating the diseased tissue into the healthy mango fruit growing on the tree, using the system utilized by Murphy and M'Kay (1926) for infecting the potato tubers with virus. By means of a cork-borer (diameter 6 mm) a small plug of healthy tissue about 10 mm in length and 6 mm in diameter, was taken out from a healthy mango fruit and was replaced by a plug of the same length taken out from the diseased portion of a fruit by the next higher size cork-borer (diameter 7 mm). The part was sealed with paraffin and vaseline mixed in a requisite proportion. All the operations were carried out in an aseptic condition. For the control, similar operations were made excepting that instead of the plug of the diseased tissue a plug of healthy tissue was inserted. The experiment was carried out only with the Safeda (Lucknow) since a sufficient number of healthy fruits was not available for the purpose of inoculation in any other variety. Altogether fifty fruits were inoculated and about the same number kept as control. The inoculation was carried on in the orchard and the fruits were allowed to remain on the tree. It was thought that any causal organism present in the diseased tissue would produce necrosis in the healthy part it was placed in contact with. No necrosis appeared. At a later stage, however, rotting due to a species of *Mucor* set in, both in the inoculated fruits and in the

controls. The organism must have come in as a contamination. More elaborate work on this aspect of the problem involving injection of healthy mango fruits with extracts from diseased tissue is in progress.

3 *Soil Condition*—It seems unlikely that the soil condition should be responsible for the incidence of the disease. The disease is recorded in diverse localities where the soil conditions are different. And in the same orchards where the soil has been specially prepared and manured for the mango tree and has thus claim to a fair degree of uniformity, different varieties behave differently towards the disease. Further, it has already been pointed out that in the Kapurthala garden there are four *Tukhm* trees, viz, Pautahia, Lambauri, Basaindha and Baheli. Of these Pautahia and Basaindha grow in a pair whereas Lambauri and Baheli form another pair. Although growing in the identical soil conditions one of each pair is very susceptible to the necrosis, the other is not at all susceptible. Thus Pautahia and Lambauri are resistant and Basaindha and Baheli are susceptible. It is possible, however, that the soil condition plays a secondary rôle in the incidence of the disease.

4 *Vigour of the Tree*—That the necrosis of the mango fruit is not due to the lack of vitality of the trees is quite apparent from our survey. Almost all the trees in the orchards under observation which showed necrosis were perfectly healthy. It was found, however, that the lack of vitality of the tree may be a contributory cause of the necrosis. For example, an unhealthy tree (tree no. 24, Bahadurpur garden, Lucknow) showed cent per cent. infection where the healthy tree of the same variety nearby had a less heavily infected crop. A similar observation was made on a variety of Safeda (Narma) where a withering tree (tree no. 6A, Bahadurpur garden) had a very poor crop of mangoes all of which were affected with the necrosis, while the mangoes on vigorous and healthy trees of the same variety nearby remained unaffected. In Zahirpur garden too an unhealthy Safeda tree showed relatively greater infection.

5 *Brick-Kiln Fumes*—It is a popular belief that the necrosis of the mango fruit is caused by brick-kiln fumes. Almost all the villagers and the gardeners in charge of the orchards attribute the disease to the fumes emitted by the brick-kilns. There seems to be some justification for holding this view, since according to them the disease follows the brick-kiln. This view is also held by many prominent persons who have sometime or other concerned themselves with the problem. For example, Woodhouse (1909) as already referred to, stated that, "The round Bhagalpur mangoes do not seem to be much affected by disease, but this year much fruit is said to have been spoilt at the Sabour Farm, by the smoke from the brick-kilns which apparently blackens the apex of the mango and interferes with its development." Also Naik (1934).

Allan (1936) is more emphatic on the effect of brick-kiln fumes on the mango fruit. He writes "There is no doubt whatsoever that the modern coal burning brick-kiln is a definite menace to a garden. Whether this damage arises as some think, from the heating effect of the kiln on the soil nearby or, as the writer believes, from the fumes emitted may not be a settled matter, but undoubtedly such a kiln specially if to the windward, i.e., to the west or south-west (between March and June) of an orchard seriously endangers the fruit which in consequence is apt to develop a blackening of the skin and a rotting tendency long before it is ripe. The presence of a kiln does not seem to affect the young tree or its growth, but comes into play when fruiting develops. The damage is in inverse rates to the distance of the kiln from the orchard boundary. To establish a garden within 400 yards to windward of modern brick-kiln is to court trouble later. To allow such a kiln to develop near a valuable garden without serious protest is again admitting trouble. If the kiln is on the leeward or side away from that of the prevalent wind, usually between March and June this means to the east of the garden, damage is much less likely and even at 100 to 200 yards but little direct harm may arise." Mr P. K. Dey, the Plant Pathologist to the Government of U.P., conducted a rough and ready experiment on the effect of SO_2 on the mango fruit by burning sulphur under a tree bearing healthy *Tukhmi* mangoes. At the time of the fumigation the fruits were about an inch in length and quite healthy without any sign of necrosis. The plant was fumigated twice a day for 7 days. On each occasion sulphur was burnt for half-an-hour in such a manner that the tree became covered with the SO_2 fumes. The exposure of the mangoes to the SO_2 fumes must have lasted much longer than half-an-hour. On the fifth day yellowish colour appeared at the tip and blackening appeared on the seventh day. Soon the diseased tissue collapsed exposing the stone and within a fortnight necrosis had claimed the distal half of the fruit. All the mangoes in the tree were affected.*

As stated, the experiment was a rough and ready one. It was limited to only one tree. No control was kept nor was the experiment carried out under controlled conditions. Nevertheless as the disease seemed to be identical to the necrosis of the mango produced by the brick-kiln fumes, investigations have been started in this laboratory to ascertain the effect of SO_2 fumes and brick-kiln fumes on the mango plant and mango fruit, particularly on the latter, under controlled experimental conditions.

During the mango season of 1937 a survey of a limited number of mango orchards in the neighbourhood of Lucknow situated by the side of Kursi

* In a private conversation with the senior author.

Road, Barabanki Road, Sitapur Road, Mahhabad Road and in Malihabad proper was undertaken. The limitation was imposed by the lack of financial resources and properly qualified surveyors at the disposal of the authors.

It appears from the above survey that the necrosis of the fruit is quite common in the immediate vicinity of the brick-kilns. It was also found in Bahadurpur orchard, which is situated at a distance of about 500 yards in a bee line from the operating brick-kiln. The Zahirpur orchard which is said to be affected by the same brick-kiln is situated at a distance of no less than 800 yards. There is no other brick-kiln nearer to Zahirpur orchards than the one mentioned above. It seems, therefore, that if brick-kiln fumes are responsible for the disease they are able to affect even at a distance of half-a-mile. A distance of 400 yards only from the brick-kiln to the orchard as suggested by Allan (1936) and Naik (1934) is clearly not enough for the protection of the orchards.

As stated, necrosis is common in the immediate vicinity of brick-kilns. But there are exceptions. In Malihabad proper Nazir Hasan Khan has a brick-kiln situated within the compound of his own orchard which has been operating for the last five years, but according to the owner none of his mangoes, which are exclusively Safeda (Malihabad), are affected by necrosis. As will be seen from Table II, Safeda (Malihabad) is susceptible to necrosis to some extent. In Kapurthala orchard only 10 per cent of the fruits proved to be diseased. If the necrosis were entirely due to the fumes one would have expected at least a few fruits in Khan's garden to be necrotic, the kiln being in such close vicinity. About two furlongs from this brick-kiln there is a very big orchard belonging to Isaq Ali Khan which has all the superior varieties of mangoes, namely, Dasehri, Safeda, etc., etc. But necrosis is unknown there. Further, there is another very big brick-kiln about 800 yards away from Nazir Hasan's orchard and separated from it by a *Nullah*. This also does not seem to have any effect on these orchards.

These observations are interesting inasmuch as they tend to preclude the possibility of the fumes being the factor producing necrosis. Even if fumes are found to be the cause of necrosis there must be other factors operating to account for the observed facts. These orchards are under further observations.

The question of the direction of the wind is closely associated with that of the fumes. If the disease is due to the fumes the importance of the direction of wind must be admitted. In the course of our survey, however, not much relation could be established between the extent of the disease and the direction of the wind. It was found that not only is there a periodic change

in the direction of the wind, but there is also a diurnal change. The wind keeps on changing its direction, so that an observer looking at a chimney top emitting fumes will see fumes being blown off in one direction at one time and in another direction sometime later. A definite direction for a prolonged period is sometimes maintained and can be seen on a still day. Alteration in the direction of the wind both at the ground level and at a height of 30 feet (chimney-top level) requires to be studied before any authoritative data can be supplied.

The three orchards affected with necrosis do not bear the same relation to the nearest brick-kiln so far as the direction is concerned. It is seen that the Bahadurpur orchard is to the east, Zahirpur orchard to the north-east and the Kapurthala orchard to the south of the nearest brick-kiln. They are all affected by necrosis.

The direction of the wind and the direction and proximity of the orchard in relation to brick-kilns are of importance inasmuch as they determine the concentrations of the fume constituents reaching the orchards. An analysis of the SO_2 content of the air at different distances from brick-kilns is in progress in the laboratory.

"Taper Tip" Disease

In the course of our investigation we came across a large number of mangoes of Dasehri variety that showed a peculiar tapering of the distal end of the fruit. On further examination it became clear that the condition could not be regarded as normal and perforce we had to come to the conclusion that it indicated a kind of disease hitherto unrecorded. The name 'taper tip' is proposed for this disease in view of the characteristic appearance presented by the affected fruit.

The external symptoms of this disease were carefully noted. It was found that the fruit of all shows an intensification of the normal green colour at the distal end. The portion thus affected apparently ceases to take part in the further development or takes part only to a very limited extent, with the result that in the mature fruit the distal end is more or less abruptly tapering (Pl II, Fig 20) and is often curved. The fruits affected by the disease fail to attain the normal size, remain relatively small and are easily detachable from the stalk. As has been already stated the taper tip disease is most abundant in the Dasehri variety. Almost cent per cent are affected in Bahadurpur orchard and a large number in orchards elsewhere. It is also found in a Bombay variety Gola-Mohanbhog, Safeda and other varieties investigated, have so far proved to be peculiarly free from this disease.

The necrosis may occur in fruits already affected with the taper tip disease. The symptoms in such cases are identical to those described elsewhere. The ætiolation of the skin associated with the necrosis makes its appearance at the distal end already affected with taper tip, which is followed by the formation of brown patches and the ultimate disintegration of the tissues.

In Bahadurpur orchard the two diseases, the taper tip and the necrosis, were so associated together that the taper tip seemed to be the earliest symptom of the incidence of necrosis even prior to ætiolation. But most probably the taper tip bears no relation to the necrosis since the disease is found to occur in the Dasehri variety in certain orchards of Malihabad and Lucknow where the necrosis is unknown. It is possible, however, that in these orchards the disease does not proceed beyond the initial stage in which case, it is to be assumed that the initial symptom of necrosis varies between varieties, for example between Dasehri and Gola-Mohanbhog.

Summary

The paper deals primarily with the external symptoms exhibited by a hitherto undescribed disease of the mango fruit. The disease has been termed *necrosis* and the popular name by which it is referred to is "Black tip" since the necrosis invariably starts at the distal end of the fruit and the necrotic area is distinctly dark-brown in colour.

The earliest external symptom of the disease is the ætiolation of the distal end of the fruit. Later on grey spots of indefinite outline make their appearance in the ætiolated area, turn brown in colour and coalesce. In the cases of more severe attack, the entire tissue thus affected collapses, exposing the stone of the fruit having a more or less thin layer of collapsed tissue over it. This is particularly prominent in Safeda, Dasehri and certain *Tukhm* varieties. The necrotic area remains restricted usually to about a quarter of the size of the fruit. The rest is not directly affected.

At an early stage of the disease, the necrotic area shows *gummosis*. The extent of exudation of the gum depends upon the variety.

Twelve orchards in the neighbourhood of Lucknow were kept under observation in the mango season of 1937. Three of these were utilised for special work relating to the disease. The result of our limited survey showed that the disease is common in orchards situated in the vicinity of brick-kilns, but not without exception.

Susceptibility to the disease varies with the variety, Dasehri being the most susceptible.

There is also a real difference in the susceptibility within a variety.

Lack of vigour of the tree seems to be a contributory cause of the incidence of the disease

It has not so far been possible to isolate any fungus or bacterium from the diseased part, neither has it been possible to induce the disease in healthy fruit by inoculating diseased tissues. Apparently the disease is not due to fungus or bacterium. The possibility of its being due to virus is still an open question.

A disease called *Taper tip* is recognised. The symptoms of the disease are that the distal end of the fruit shows an intensification of the normal green colour, tapers abruptly and is often curved, causing the affected fruit to remain smaller than the average ones.

The authors wish to express their thanks to Mr P. K. Dey, the Plant Pathologist to the U.P. Government, for the information relating to the disease. Their thanks are also due to Sheikh Abdur Rahman, Mr. Devi Shanker Shukla, the Kapurthala State, and other orchard owners who gave facilities to carry out investigation in their respective orchards and to Prof. B. Sahni, Sc.D., F.R.S., for taking a keen interest in the investigation and also for his valuable suggestions and criticism.

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DESCRIPTION OF PLATE FIGURES

FIGS. 1-12 *Dasehri variety*

- FIG. 1.—A normal, healthy mango of *Dasehri* variety
- FIG. 2.—Earliest symptom. etiolation of the skin of the apical region
- FIG. 3.—Intensification of etiolation.
- FIG. 4.—Appearance of brownish spots in the etiolated area
- FIG. 5.—Spots larger and dark-brown; earlier stage of coalescence
- FIG. 6.—Formation of continuous necrotic area due to the coalescence of the dark-brown spots.

Figs. 7-9.—Necrosis more advanced. An etiolated zone round the dark-brown necrotic area

Figs. 10-12 —Stages in the emergence of seed owing to the collapse of healthy tissue due to *necrosis*. The zone of etiolation round the necrotic area is still persistent

FIG. 13.—*Safeda variety*. A mango showing advanced stage of *necrosis*. Seed protruding and a faint etiolated zone round the necrotic area is visible.

Figs 14-19.—*Gola-Mohanbhog variety*

FIG 14 —Earliest symptom etiolation of the skin of apical region.

FIG 15 —Appearance of small brown spots in the etiolated area.

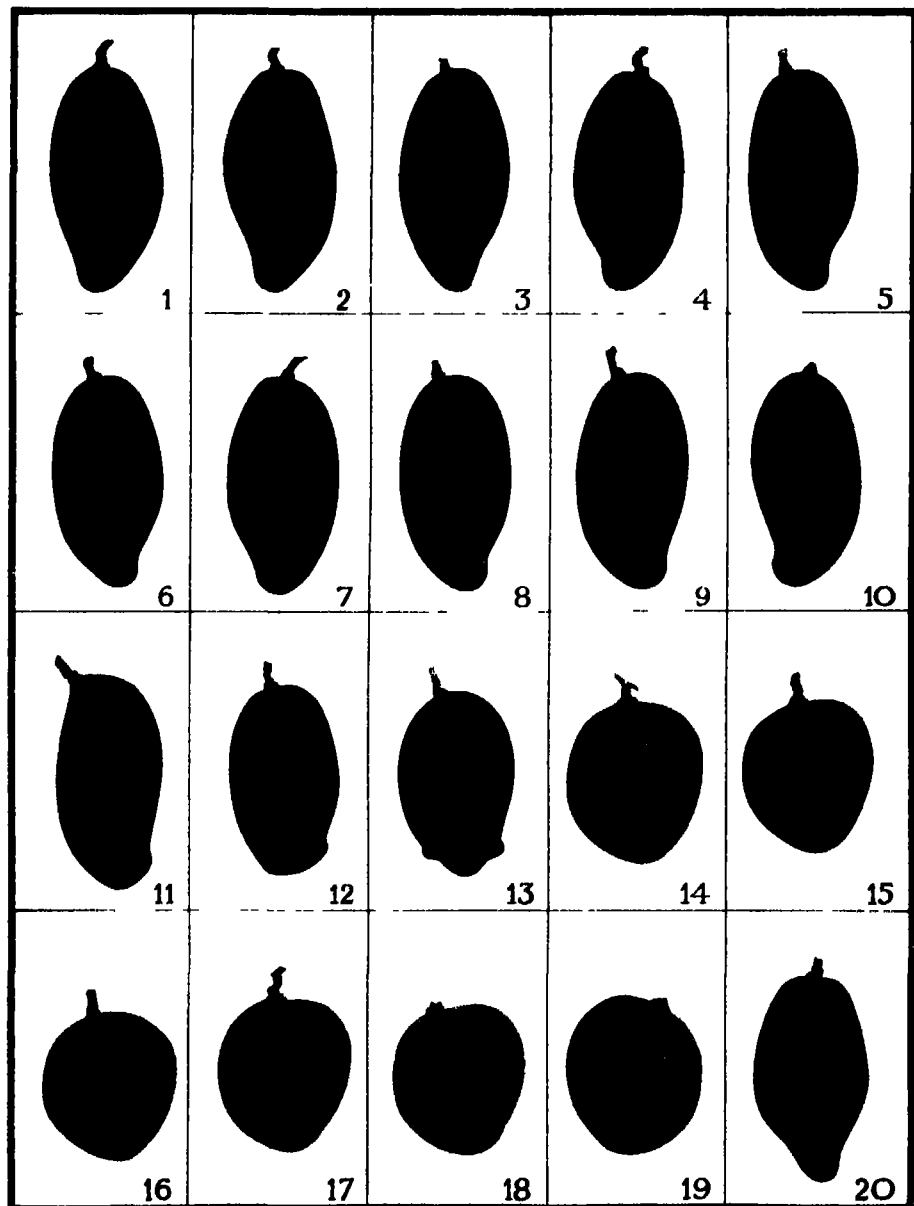
FIG 16 —Spots larger and in the earlier stages of coalescence

FIG 17 —Formation of continuous necrotic area due to coalescence

FIG 18 —More advanced stage

FIG. 19.—The most advanced stage so far found in Gola-Mohanbhog

FIG 20.—A mango of *Dasehri* variety showing the *Taper tip* disease.



A PRELIMINARY ANALYSIS OF PANICLE STRUCTURE IN SORGHUM—THE GREAT MILLET

BY G N RANGASWAMI AYYANGAR, F N I, I A S
Millets Specialist and Geneticist, Agricultural Research Institute
AND

D S RAJABHOOSHANAM, M A
Assistant, Millets Breeding Station, Coimbatore

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AMONG cereals, sorghum is remarkable for its wealth of panicle types. They answer to the description loose, compact, cylindric, conic, ellipsoid, oblong, lanceolate, ovoid, truncate, etc. With a view to give a metrical preciseness to these descriptive terms used in the course of the pursuit of the fundamental problem of yield, 39 panicles, belonging to 18 species of sorghum, were analysed into their components (Table I).

A panicle is what it is by the manner in which the metrical components combine. These are described below.

1 *The length of the rachis*—This is measured from the bottom whorl to the topmost one (Fig. 1). The rachis varies in length from 1.5 cm. in

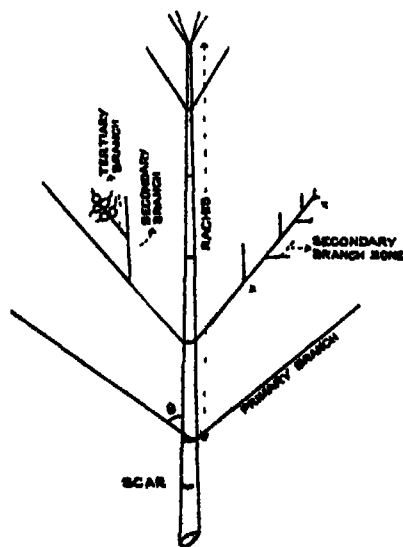


FIG. 1

The Components of a Sorghum Panicle

TABLE I
Summary of Panicle Measurements in Sorghum

Selection Number	Botanical species	Length of rachis	Number of whorls	Number of primary panicle branches	The secondary branch zone on the primary branch in the first whorl	The index of spikelet clustering in the first whorl	Total number of fertile spikelets	Weight of 100 grains	Type of disposition of primary branches
A.S. 4297	<i>S. marginiferum</i>	cm 34	16	55	%		1075	gm 1.2	I
" 4367	<i>S. guineense</i>	30	20	99	70	1.5	2133	4.2	I
" 4674	<i>S. complanatum</i>	16.5	5	36	70	1.9	3826	1.8	III
" 4639	<i>S. Roemerianum</i>	13	12	59	70	2.7	3573	2.4	IV
" 4092	<i>S. exaristatum</i>	50.5	14	94	60	1.3	2135	4.6	I
" 2876	<i>S. nervosum</i>	19.8	9	36	80	2.1	1048	2.1	I
M.S. 3418	<i>S. "</i>	17.1	14	130	90	3.0	4111	2.2	II
A.S. 3782	<i>S. malabaricum</i>	18.3	9	75	90	2.4	3109	2.6	I
" 5157	<i>S. splendens</i>	30	8	33	60	2.8	1778	2.2	I
" 1139	<i>S. dachin .</i>	3		60	47	1.2	3164	2.0	IV
M.S. 3301	<i>S. "</i>	3	4	23	60	0.7	716	2.1	IV
" 3294	<i>S. "</i>	52	8	77	60	1.4	1346	1.6	I
A.S. 2066	<i>S. "</i>	22	14	103	54	4.8	2374	1.3	I
" 3284	<i>S. "</i>	18.4	10	97	70	3.1	2177	1.4	II
" 3283	<i>S. "</i>	25.2	17	87	90	4.4	1648	1.6	I
" 3356	<i>S. bicolor</i>	18	12	90	60	2.0	3287	1.9	II
" 3464	<i>S. cafferum</i>	12	11	58	..		2447	1.9	III

" 4003	S "	21	9	72	40	8 0	3814	3 3	II
" 4009	S "	13	12	159	80	5 1	3807	3 0	II
A.S. 3998	S <i>rugosius</i>	1 5	3	15	60	23.2	5233	1.2	IV
" 4049	S "	8 6	8	41	80	8 9	7694	0 8	IV
" 4052	S "	25	16	66	80	3 0	2575	1 7	I
" 4053	S "	14.6	14	45	70	12 0	2270	2 5	I
" 4056	S "	18 4	14	80	70	3 7	2818	3 0	I
" 3400	S <i>conditum</i>	21 0	18	52	83	3 0	1265	4 7	I
" 3981	S "	14 7	12	45	70	6 2	2420	2 0	III
" 4540	S "	18.1	9	41	90	2 1	2224	4 3	III
" 29	S <i>durr</i>	18	13	102	80	2 8	3436	2 7	II
" 3785	S "	13 5	18	144	54	5 2	1973	6 1	II
M.S. 3398	S "	11.4	6	118	80	2 6	3543	5 4	II
T 1	S <i>ocimum</i>	6 5		121	77	3 6	3437	3 4	II
A.S. 367	S <i>subglaberrime</i>	8	12	85			1990	2 6	III
" 809	S "	10 6	9	78	70	4 9	2391	3 2	II
" 3684	S "	18 4	11	53	90	5 0	1565	3 0	II
" 4996	S "	14 2	9	76	90	4.3	3476	2 9	III
" 4999	S "	9 4	11	108	70	7 3	3152	2 0	III
" 5000	S "	10 7	12	111	60	7 5	3376	2 8	III
M.S. 3338	S <i>seductum</i>	13 7	8	32	90	3 7	291	0 6	I
" 3336	S "	26	12	45	80	1 2	981	0 3	I

Karawiru, a variety of *Sorghum nigricans*, to 52 cm in other conical earheads. In the crosses made at this Breeding Station, the long rachis is found to be dominant to the short one.

2 *The number of whorls on the rachis*—Distinct whorls of branches are discerned clearly in a panicle with a long rachis, the spacing between the whorls being then well marked. In panicles with a short rachis, the whorls tend to be spiral. The number of whorls may be as many as 20.

3 *The degree of panicle branching*—From the rachis arise primary panicle branches, and from these arise secondary panicle branches and these ramify into tertiary branches. Generally in the lower whorls of a panicle, spikelets are borne on the tertiary branches and towards the top whorls, on the secondary branches. The exceptions to these are met with in a type of Sudan grass where the ramification of the branches stops with the secondary branches and in *Karawiru* (*S. nigricans*) where it extends up to the fourth degree of branching.

4 *The length of the primary panicle branch and its disposition on the rachis*—From the first to the last node, each primary branch in a panicle was measured for its length. The maximum length of the primary branch in a panicle tends to have a relationship with the length of the rachis in the different species of sorghum. The correlation between these two factors is .78, a significant value. Two notable exceptions are *Karawiru* (*S. nigricans*) and broom-corn (*S. dochma*). The branches are of almost the same length in the same whorl, but vary in different whorls according to the following four patterns (Fig. 2).

Type I is characterised by a steady decrease of the primary branches from the first to the last whorl at the top. Conical panicles are the result of this mode of variation. *S. margaritifolium* and *S. guineense* are examples of this. In Type II, the panicle branches increase in length in the first few whorls and then decrease. Ovoid, ellipsoid and obovate panicles are the result of this type of variation of the primary branches. This type is met with in *S. caffrorum* and *S. durra*. In Type III, the length of the primary branch fluctuates within narrow limits, from whorl to whorl. Cylindric panicles arise from this mode of behaviour of the panicle branches. An example of this is *Chamziango*, a variety of *S. caudatum*. The length of the primary branches, in Type IV, increases steadily from the first to the last whorl. This mode of variation produces the truncate and corymbiform panicle. Some varieties of *S. nigricans*, broom-corn (*S. dochma*) and *Talai-virichan* (*S. Roxburghii*) are typical of this group.

that the evolution and fixity of these well-established agronomic types have a definite panicle background

5 *The angle made by the primary branches of the first whorl with the rachis*—This angle usually diminishes from the first whorl towards the last. It is determined by the degree of development of the pulvinus, and is found to vary from 0° in *ε*-ligulate plants due to the absence of pulvinus up to 90° , most frequently found in the wild Sudan grass. When the length of the primary seed branch is out of proportion to its rigidity, the branches which are erect at the time of anthesis, begin to droop by the weight of the maturing caryopses, leaving the short branches higher up on the rachis erect, and presenting the characteristic appearance of *S. Roxburghii*.

6 *The total number of primary branches*—This number varies from 15 in *Karawiru*, a variety of *S. nigricans*, to 159 in *S. caffrorum*. It does not depend upon the length of the rachis. The long panicles are likely to produce as many branches as the short ones. The correlation between the length of rachis and the total number of branches is .05 which is quite trivial.

7 *The secondary branch and its relationship with the primary branch*—On each primary branch, the length of the first secondary branch nearest the rachis was measured. The relation between these two lengths is represented in most of the species by the straight regression line

$$y = .25x + 12.7$$

where y is the length of secondary branch and x that of primary branch. In this, the first secondary branch is roughly $\frac{1}{4}$ the length of the primary branch on which it is borne. There is an indication of a second trend defined by the line

$$y = .12x + 1.8$$

In this group, the first secondary branch is roughly $\frac{1}{8}$ the length of the primary branch on which it is borne.

Sufra (a *S. durra* from the Sudan) and *S. conspicuum* do not conform to either of these regressions.

8 *The secondary branch zone of the primary branch*—That fraction of the primary branch which bears the secondary branches varies in different species of sorghum. Considering the first whorl only for a standard comparison, the proportion of the secondary branch zone to the total length of primary branch varies from as much as 90 per cent in many species to 54 per cent in *Irungu* (*S. dochna*) and 47 per cent in broom-corn (*S. dochna*). The *ε*-ligulate condition definitely increases the secondary branch zone and results in a congestion of spikelets (Rangaswami Ayyangar, *et al.*, 1938). In an F_2 progeny (A S 3699) belonging to the group *S. dochna*, and

segregating for the ligulate and *s*-ligulate condition, the secondary branch zone in the ligulate group was 60 per cent. and in the *s*-ligulate group 86 per cent. The corresponding figures are 84 per cent and 100 per cent in a segregating progeny (X-18) belonging to the group Sudan grass

9 *The index of clustering of fertile spikelets*—This index on a primary branch, may be defined by

$$D = NL/nl',$$

where N is the total number of fertile spikelets, L, the length of the primary branch; n, the number of secondary branches on the primary branch, l, the length of first secondary branch, L' the secondary branch zone on the primary branch. To standardise comparison, the index D is measured in the primary branches of the first whorl. *S. nigricans*, shows generally a high index (3 to 9). Of these *Karawuru* with its small grains, has an exceptionally high index of 23 due largely to the fertility of its pedicelled spikelets. The least index is in broom-corn (*S. dochna*), which has a value of only .7 and hence eminently a broom

With an increase in the quantity N/nl , which is a factor in D, and a shortening of the tertiary branches and the clustering of the spikelets towards the terminal of the secondary branch a spurred condition of the earhead contour results. This is met with in *S. nigricans* and the compact earheads of irrigated sorghums belonging to the group *S. subglabrescens*

10. *The weight of the grain*—The weight of the grain has been found to be highly correlated to its size (Rangaswami Ayyangar, *et al*, 1938), so that weight is an index to the size of the grain. From each panicle analysed, 100 grains picked at random were weighed. The largest and heaviest grain (61 mgm) is in *Sufra* (*S. durra*) and the weight per grain decreases to 12 mgm. in *S. margaritifera* and 3 mgm. in *S. sudanense*

The weight and therefore, the size of the grain, has practically nothing to do with the total number of fertile spikelets, the correlation between these two factors being —.24 which is not statistically significant. A large number of grains does not necessarily mean a diminution in the weight of the individual grain

The weight of the individual grain plays an important part in the problem of yield (Rangaswami Ayyangar, *et al*, 1935)

11 *The total number of fertile spikelets*.—This in itself has no fixity, but is conditioned by the total length of primary branches and density of clustering of spikelets. In the wild *S. sudanense* the number of spikelets is as low as 290, 2,000 to 3,000 spikelets is the common number in the average grain sorghums. A variety of *S. nigricans* (A.S. 4649), due to the smallness of

the grain and the pedicelled spikelets also being ovariferous, gave as many as 7,700 fertile spikelets

The total number of fertile spikelets does not depend upon the number of primary branches, the correlation between these two factors being .15. But it depends on the sum of all the lengths of the primary panicle branches which is the total effective region for bearing spikelets. The relation between these factors depends on the linear density of clustering of spikelets, i.e., the density per unit distance. On plotting the points in a diagram (Fig 3) three trends become apparent, (1) dense, (2) normal, and (3) sparse

In the dense class the trend of the relationship between the number of fertile spikelets, denoted by y , and the total lengths of the primary branches, denoted by x , can be expressed by the straight regression line

$$y = 26.41x - 266.8$$

S. nigricans belongs to this class. The normal class is described by the line

$$y = 3.44x + 1062$$

The corresponding correlation is .61, which is significant. To this belong most of the economic grain sorghums. Lower down in the diagram (Fig 3).

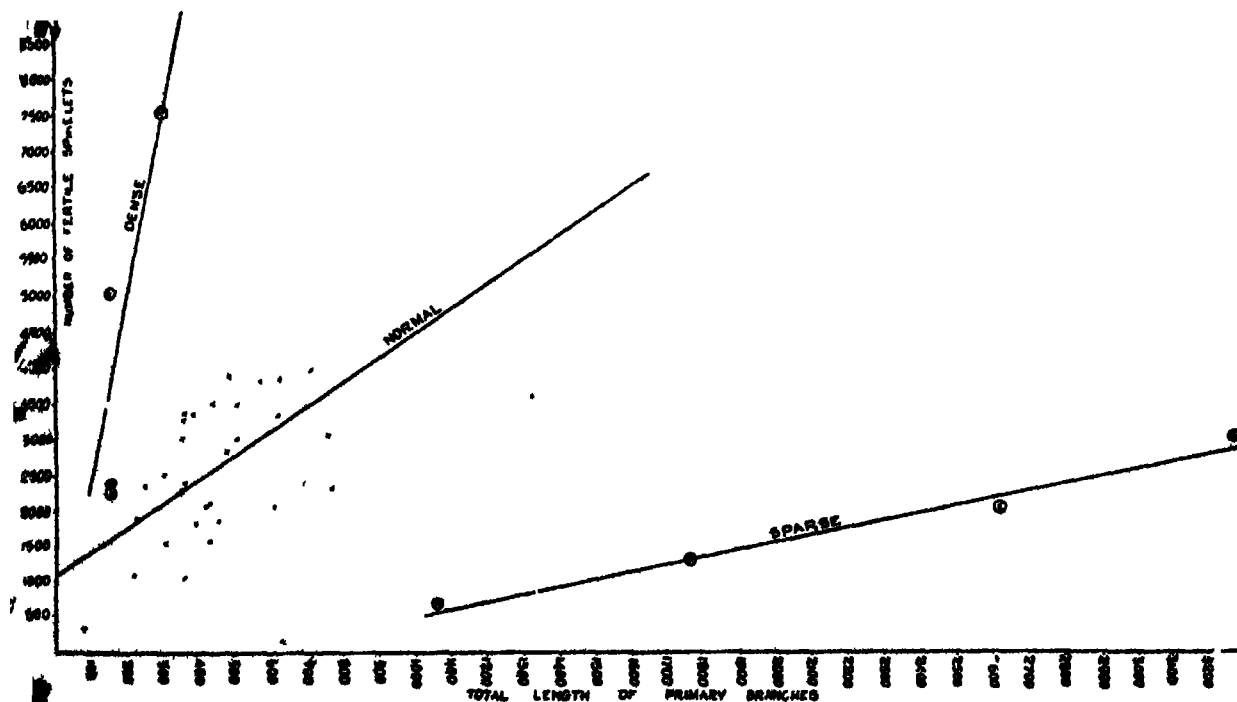


FIG. 3

Sorghum—Total Length of Primary Branches and the Number of Fertile Spikelets

the trend of the sparse type is apparent This may be represented by the regression line

$$y = 1.06x - 668$$

Broom-corn (*S. dochna*) and *S. exsertum* belong to this type The coefficients of x , namely 26.41, 3.44 and 1.06, indicate the dense, normal and sparse linear density The blending of these two factors, *viz*, the weight per grain and the condition determining the number of fertile spikelets in a sorghum earhead, is the problem in yield

Summary

The importance of studying the ultimate components of the panicle when engaging on the problem of yield is emphasised The different types of panicles in sorghum are shown to arise from different possible combinations of the factors, length of rachis, the mode of variation of the primary panicle branches, the extent of ramification of panicle branching, the angle which the whorls make with the rachis, the secondary branch zone of the primary branch, and the density of clustering of fertile spikelets Relationships are described between length of rachis and 4 types of panicle, length of rachis and maximum length of primary branch, number of primary branches and length of rachis, length of primary branch and secondary branch, total number of fertile spikelets and weight per grain, total number of fertile spikelets and the sum of the lengths of all the primary branches The solution to the problem of yield must be based on the weight and size of an individual grain and the components which determine the number of fertile spikelets

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A CYTOLOGICAL STUDY OF *SCAEVOLA* *LOBELIA* LINN.

By S B KAUSIK

(From the Department of Botany, Central College, Bangalore)

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Introduction

THE literature in the family Goodeniaceae as reviewed by Schnarf (1929, 1931) reveals that only two members, *Scævola Kœnigii* and *Dampiera stricta* have been investigated. The work of Billings (1901) on *Scævola* treats of the embryology of the plant, while that on *Dampiera* by Brough (1927) relates to the development of pollen and the formation of the embryo sac. A detailed cytological investigation does not seem to have been undertaken in the family so far.

The material for the present study, in which a detailed examination of the important stages in the meiotic cycle of the microspore mother cells is made, was collected during October 1937 in the island of Krusadai, near Pamban, South India. There are only a few plants on the sandy shore of the island, well above the high tide mark and about a furlong from the Marine Biological Laboratory. The plants are small in stature and thick-set with large succulent leaves. The flowers are conspicuous with a bright yellow and fleshy corolla, which is strongly zygomorphic.

The material was killed in Bouin's fluid and subjected to the usual course of treatment. Sections were cut varying in thickness from 8 to 10 μ and stained in Heidenhain's iron alum hæmatoxylin.

Structure of the Anther

The wall of the anther shows an epidermis, an endothecium, a single middle layer and the tapetum (Fig 2). All the layers within the epidermis are formed, as usual, from a primary parietal layer, which in itself is derived from the hypodermal archesporium at an early stage. After the separation of the wall layers, the sporogenous cells appear in the form of four greatly elongated cylindrical masses at the four corners of the anther (Fig 1). The sporogenous cells are sharply separated off from the tapetal cells by the presence of dense cytoplasm and large conspicuous nuclei. The tapetal cells are uninucleate to begin with and contain one or more vacuoles. As the nuclei of the microspore mother cells approach the diplotene stage in meiosis,

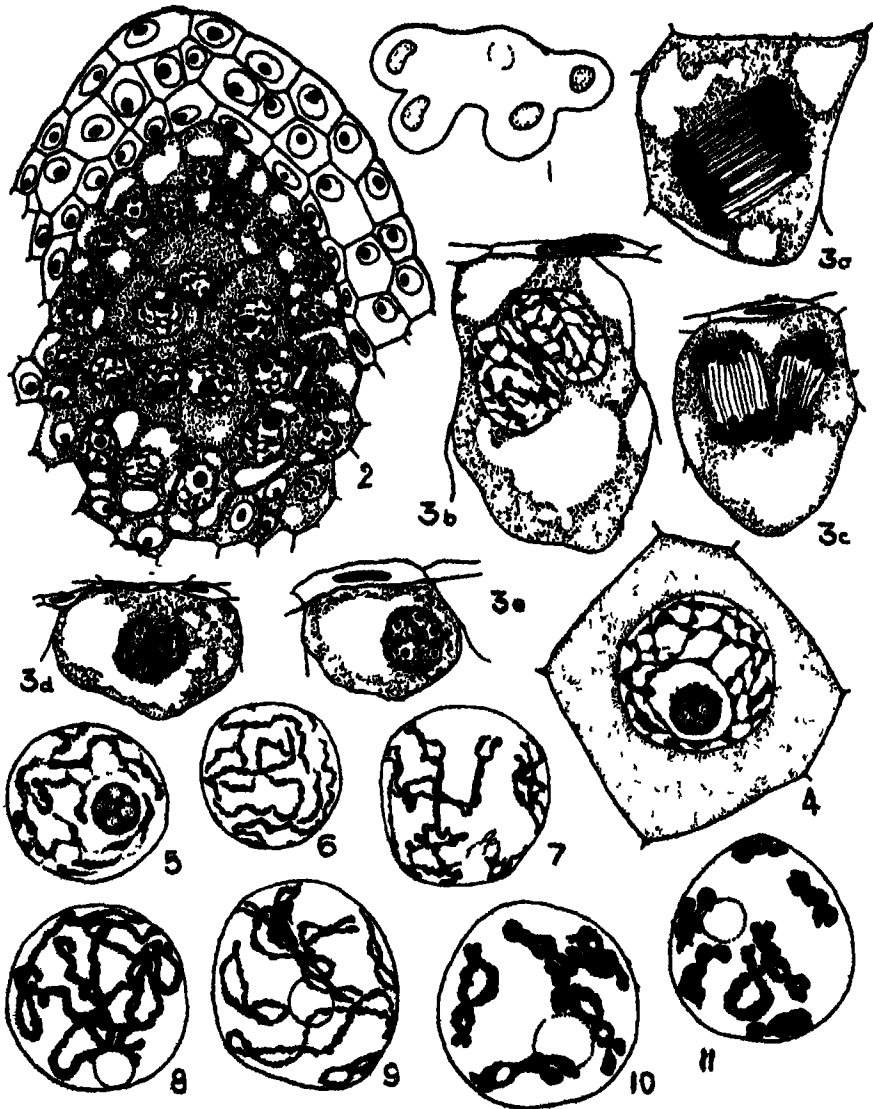
the nucleus of each tapetal cell divides once mitotically to form two nuclei (Fig 3a and b). These nuclei divide again for the second time a little later (Fig 3c), so that the tapetal cells become quadri-nucleate. Sometimes, however, only three nuclei, one large and two small, may also be formed if the disposition of the spindles of the second division is such that the poles are near each other and the result is the inclusion of the daughter chromosome groups within a common nuclear membrane. The tapetal cells finally become uninucleate again by a fusion of all the free nuclei just prior to the decline in the tapetal activity (Fig 3d and e).

In addition to the normally formed tapetal cells derived from the primary parietal layer described above, some of the sporogenous cells also seem to take part in tapetal function to some extent. Such sporogenous cells are found towards the inside of the sporogenous groups, namely, next the connective tissue of the anther (Fig 2). These cells are clearly seen to be arrested sporogenous cells to serve a tapetal function. They are not only continuous with the regular sporogenous cells but also have large and conspicuous nuclei, which are practically in the same stage of development as those in the sporogenous cells.

Meiosis in the Microspore Mother Cells

The resting nucleus of the microspore mother cell shows a number of large and darkly staining chromatin masses, which are connected to one another by fine and delicate threads (Fig 4). With the first sign of meiosis, the chromatin masses become elongated and resolve themselves into a number of threads of different lengths (Fig 5). The delicate net-like connections are still visible, but become gradually lost as the leptotene stage is reached. The leptotene threads are uniformly distributed inside the nuclear membrane without showing any definite orientation. The threads appear to be beaded with clear diamond-shaped areas in certain portions (Fig 6).

The leptotene threads next show evidences of coming together in pairs for synapsis, which marks the zygotene. The pairing of the threads seems to commence anywhere along the length of the threads, but is invariably clearer at the ends (Fig 7). A distinctly double nature marking the chromonemata is seen in particular regions of the threads which have not as yet synapsed. With the completion of zygotene pairing, the threads become uniformly thick and intensely staining to give rise to the typical pachytene stage (Fig 8). The four-partite nature of these threads becomes obscure, and a "bivalent" nature only, and that in certain portions alone, can now be seen. Further, the pachytene stage is characterised by a definite orientation of the threads with reference to the nucleolus, though intimate connections between the two are at no time evident.



FIGS 1-11

Fig 1—Outline of transverse section of anther $\times 80$ Fig 2—Part of anther to show wall layers, tapetum and the microspore mother cells. Note that the mother cells towards the inside appear tapetum-like. $\times 800$. Fig. 3 a, b, c, d and e—Tapetal cells at different periods of their activity (explanation in text) a, b and c $\times 1800$; d and e $\times 900$. Fig 4—A microspore mother cell with its nucleus in resting condition $\times 2700$. Fig 5—Resolution of the net-work of the nucleus to form the threads $\times 2700$. Fig. 6—Leptotene showing the coiled chromomemata forming diamond-shaped areas $\times 2700$. Fig. 7—Early zygotene, synapsis is taking place in certain portions. $\times 2700$. Fig. 8—Pachytene. Note orientation of threads and the "bivalent" nature. $\times 2700$. Fig. 9—Early diplotene $\times 2700$. Figs 10 and 11—Mid-diplotene nuclei showing the full haploid set. $\times 2700$.

The emergence of the threads from the pachytene stage next becomes manifest by the falling apart of parts of the synapsed threads (Fig. 9) except at regions where the chiasmata are established. It is not possible to determine exactly where the chiasmata are first established. The formation of the chiasmata in all the pairs gives rise to the diplotene stage. Here again a four-partite nature becomes once more evident in some regions.

The number and nature of the chiasmata in the diplotene pairs depend largely on the relative lengths of the pairs themselves (Figs 10 and 11). Some of the pairs, usually four or five and which are slightly longer than the rest, have three chiasmata each, of which one alone is terminal and the others are interstitial in some long pairs, while all the three chiasmata are interstitial in the other long pairs. The short diplotene pairs have only two chiasmata, one terminal and the other interstitial. A single short pair, however, seems to have a single terminal chiasma from the earliest clearly recognizable condition. This pair is evidently the first to show a separation of its components at the first anaphase (Fig 17).

The total number of chiasmata formed in all the pairs in a mid-diplotene nucleus is found, from an examination of a number of nuclei, to be approximately twenty, of which nearly 50% are terminal. In slightly earlier stages of diplotene, the proportion of terminal chiasmata is less, namely, about 33%. In late diplotene the total number of chiasmata is reduced to the minimum of fifteen which are completely and regularly terminalized.

Structure of the Chromosomes

The late diplotene pairs reveal that each homologue is composed of two strands which are coiled in opposite directions (Fig 14, *a*, *b* and *c*). Ordinarily, the two-coiled strands appear like a number of darkly stained cross bars in a less darkly stained matrix. The chromosomes, therefore, have a wavy outline. When, however, the chromosome pairs are examined more critically the coiled nature of the strands composing the homologues becomes evident, especially in some of the pairs which have been a little extra destained or have become pale on account of time. Straining a little further, it is possible to make out in such pairs that the coils possess points of reversal usually situated on either side of the chiasmata. Since, however, the chromosomes are not large it is not possible to state definitely whether changes in the direction of coiling are restricted or are found at random in the homologues. The coiled nature becomes obscure in the first metaphase chromosomes, as these are very small and appear intensely homogeneous.

There is usually a single nucleolus in the nucleus of the microspore mother cell. This shows in the earlier stages of prophase a number of clear spaces, which become less evident later and finally completely disappear from view.

with increased affinity of the nucleolus to the stain. With progressive prophasic transformations of the nucleus, a corresponding reduction in the size of the nucleolus is seen. When the nucleolus is seen for the last time before completely and suddenly disappearing at late diakinesis, it reaches its minimum size and no trace of it is left when the chromosome pairs are arranging themselves on the equator of the first metaphase spindle. It does not reappear in the interkinetic nuclei at the conclusion of the first division, but becomes evident again only in the nuclei of the microspores after the second division is over.

The haploid number of chromosomes was found to be eight in a number of first metaphase plates (Fig 16). This number also corresponds to the number of pachytene threads and the number of diplotene pairs examined in favourable preparations. The counting of the chromosome number was also checked in the first anaphase and second metaphase stages for purposes of confirmation.

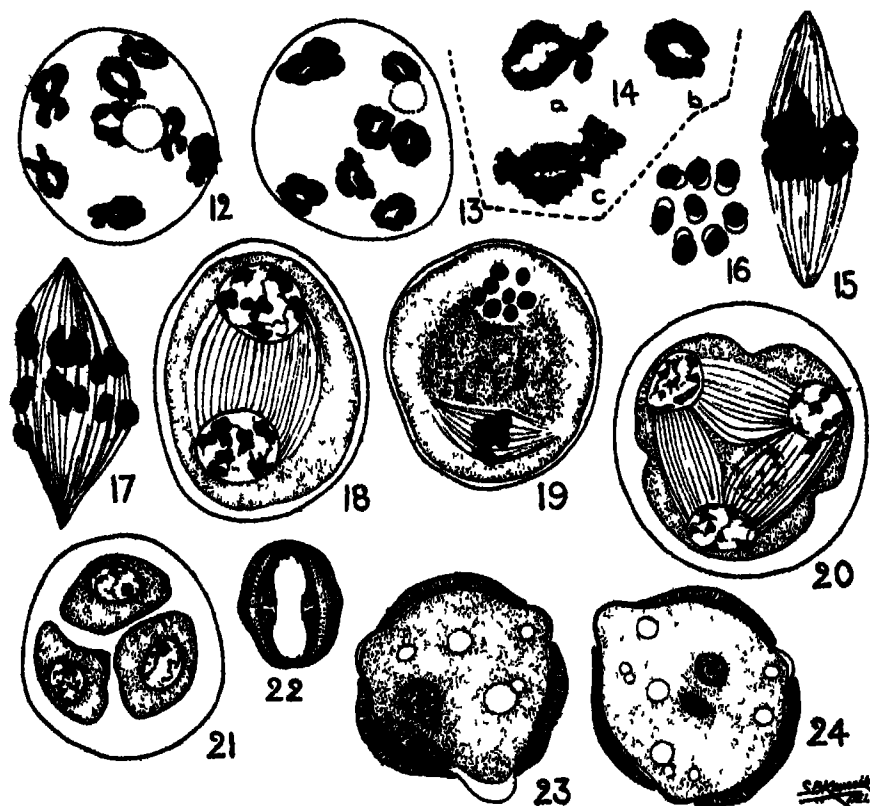
After the chromosomes have taken their positions on the equator of the first metaphase spindle (Fig 15), the anaphase separation begins (Fig 17). The homologues of one pair appear to move away from each other earlier than those of the other seven pairs. This pair showing the early separation is evidently the one characterized by the possession of a single chiasma in the diplotene stages. After the separation of the homologues of the other pairs is also effected and on the daughter chromosomes reaching the poles the interkinetic nuclei are formed (Fig 18). These nuclei do not seem to undergo any period of rest, but become ready immediately for the second division which soon follows (Fig 19).

During the second division, the two spindles lie at right angles to each other (Fig 19) so that the resulting nuclei are arranged in a tetrahedral manner (Fig 20). A dense zone of cytoplasm occupies the region of the mother cell between the two spindles (Fig 19), this zone marks the position of the first division spindle.

After the second division is over, the mother cells form quartets of nuclei connected together by spindle fibres (Fig 20). These spindles do not take part in the separation of the microspores, which is effected by the formation of advancing peripheral furrows. The separation of the microspores is simultaneous as in a number of cases recorded in literature (Schürhoff, 1926, Schnarf, 1929, 1931). The young microspores are free inside the original mother cell wall (Fig 21). The latter undergoes gradual dissolution and the microspores are set free in the anther locule as distinct pollen grains.

The pollen grains are spherical in outline and possess a thick exine. The intine is thin and bulges out at the regions of the germ pores which are

three in number When mature, each pollen grain has a tube nucleus and a generative cell lying towards one side (Fig 23) The plasma sheath of the



FIGS 12-24

Fig 12—Late diplotene $\times 2700$ Fig 13—A diakinesis complement showing completely terminalized chiasmata $\times 2700$. Fig 14 a, b, and c—Diplotene pairs showing the spiral nature of the strands $\times 3600$. Fig. 15—Metaphase in profile $\times 2700$ Fig 16—A metaphase plate showing the haploid set of eight chromosomes $\times 2700$. Fig 17—First anaphase; chromosomes of one pair, on the extreme left, have moved away earlier than those of the rest $\times 2700$ Fig 18—Interkinesis. $\times 1800$ Fig 19—Second division; eight chromosomes are seen in the upper division figure $\times 1800$ Fig 20—Formation of the four daughter nuclei and the peripheral furrows $\times 1800$ Fig 21—Young microspores within the original mother cell wall. $\times 1200$ Fig 22—A pollen grain showing the elongated germ pore. $\times 600$. Figs. 23 and 24—Mature pollen grains showing the binucleate condition. In Fig 23 the cytoplasm of the generative cell is seen. $\times 900$

latter becomes obscure a little later when the pollen grain is actually liberated from the anther locule (Fig 24) Brough (1927) states that the pollen grains of *Dampiera* are also two nucleate at the shedding stage.

The wall of the mature anther consists of the same original layers. The endothecium develops fibrillar thickenings. The tapetum is completely disorganized and the single middle layer of the wall of the anther gets usually crushed between the endothecium and the developing pollen grains.

Discussion

While convincing evidence has accumulated in an overwhelming majority of the angiosperms for pointing out the derivation of the anther tapetum from the primary parietal layer, instances are not entirely wanting to indicate the formation of tapetum from the sporogenous cells. Coulter (1898), from a study of a number of species of *Ranunculus*, states that the tapetum is sometimes derived from the sporogenous tissue and sometimes from the primary parietal layer. Singh (1936), in a recent study of *Ranunculus sceleratus* regards that Coulter was perhaps led to conclude that the tapetum is "occasionally derived by the sterilization of the sporogenous cells" on account of a great similarity between the tapetal and the sporogenous cells in their contents and staining reactions. He further remarks "During recent years the tapetum is shown to have a parietal origin in so many angiosperms that any claim of a different origin will have to be strongly supported."

Joshu and Venkateswaralu (1936), in their studies in the *Lythraceæ*, state that the "tapetum seems to be derived largely from the outer sporogenous cells." In support of this the authors present two figures (cf 75 and 76). They do not, however, clearly state whether all or only a part of the tapetum is formed thus and also, whether any process of conversion or sterilization of sporogenous cells takes place.

In *Scævola Lobelia*, the tapetum is, however, normally formed from the parietal tissue, but in addition, a further contribution to tapetal function is made by some of the sporogenous cells, those lying on the inside next the connective tissue. These, as already noted, lose their characteristic features and become tapetum-like both in appearance, as well as in function. These cells may therefore be regarded as arrested sporogenous cells which become subjected to a process of sterilization at some time during the early prophase. They thus constitute a secondary tapetum and the statement of Coulter (1898) that the tapetum may occasionally be derived by sterilization may not be wholly wrong.

In *Zostera*, according to Rosenberg (1901), the tapetum can be traced back to the divisions of the greatly elongated sporogenous cells on both the inner and the outer sides of the anther locule. Wylie (1904) states that in *Elodea* "the divisions of sporogenous cells on the axial side seem to be of common occurrence and there is probably a regular contribution to the

tapetum from the sporogenous cells in that region. In addition there may be a contribution to the diffuse tapetum on any side by the sacrificing of potential spore mother cells to the nutritive function."

Gates and Rees (1921) find that in *Lactuca* a "peculiar feature which is associated with the loose arrangement of the tapetal cells is the fact that a variety of transitional stages occur between tapetal cells and the pollen mother cells." The authors further remark that some of the tapetal cells are "scarcely distinguishable from pollen mother cells except by their position. The presence of such transitional forms between pollen mother cells and tapetal cells probably accounts for the occasional occurrence of synizesis in such cells." They, however, do not state whether an actual conversion of spore mother cells to a tapetal function takes place. On the other hand Maheshwari (1934) finds in *Ophiopogon* that "all the sporogenous cells do not reach the mother cell stage. Some are abortive and may serve to nourish the remaining cells." This indicates clearly that in addition to a normal tapetum, a tapetal function may also be taken over by some of the sporogenous cells. It is of interest to note here that a tapetal function may sometimes be ascribed even to the cells of the middle layers of the anther (Coulter and Chamberlain, 1903, Singh, 1936).

The presence of more than a single nucleus in the tapetal cells has been shown in a number of angiosperms. Cooper (1933), after a careful analysis, classifies the tapetal cells into three broad groups: (1) those in which the nucleus rarely, if ever, divides and the cells remain uninucleate, (2) those in which the nucleus divides mitotically once at about the time when the neighbouring pollen mother cells are in synizesis and the tapetal cells remain binucleate thereafter, and lastly (3) those in which the nucleus first divides as in the second group, after which further mitoses may occur resulting in plurinucleate cells. The tapetal cells in *Scævola Lobelia* become quadri-nucleate usually, but sometimes are trinucleate on account of an irregular disposition of the spindles. The nuclei subsequently fuse again to form uninucleate cells just before the tapetal activity begins to decline. Raghavan (1938) has shown that a common condition of the tapetal cells in *Gynandropsis pentaphylla* is to form four nuclei which fuse subsequently.

Several authors have described the leptotene threads as double and presenting a beaded appearance on account of the intertwining of the two chromonemata. Koshy (1937) regards that diamond-shaped areas seen in favourable portions of the leptotene threads are suggestive of this feature. Naithani (1937), however, dismisses this for what appears to be an artefact caused by the fixing fluid; he states that in iron aceto-carmin preparations the leptotene threads are single and finely coiled in *Hyacinthus*. Similarly,

Huskins and Smith (1936) do not find double leptotene threads in *Trillium*, a linear arrangement of chromosomes is, on the other hand, evident here

It is not possible to say definitely whether the leptotene threads in *Scævola Lobelia*, which present a beaded appearance and in which the clear diamond-shaped areas are seen, are really double with the two chromonemata coiled together, or, whether the picture obtained is merely an optical illusion induced by the fixing fluid. Unfortunately, other fixing fluids than Bouin's could not be employed at the time when the material was secured for study

The structure of the chromosomes and the existence of spirally coiled strands composing them have been topics of much discussion recently. In specially favourable materials the precise nature of the major and the minor coils have been convincingly shown. It is not proposed to enter here into a discussion of this interesting aspect of study, because, the chromosomes are small and hence unsuitable for a detailed examination. It must suffice for the present to state that indications of a spiral nature are seen in some late diplotene pairs, which have lost the extra stain, either by continued destaining or on account of time. Further, the chromosome outline itself is very wavy suggesting the possibility thereby of the existence of coiled strands

Darlington (1937) points out that two major types in the process of terminalization of chiasmata may be recognized. These are (1) the *Fritillaria*-type with slight change of position and little or no reduction in the number of chiasmata through fusion, and (2) the *Campanula*-type, in which the movement is complete and results in terminalization of all the chiasmata. The plant under study here thus belongs to the second category, the chiasmata being regularly and completely terminalized towards late diakinesis. No irregularities are, therefore, seen in any of the pairs during the first anaphase separation

Summary

1 The paper is a detailed account of the prophasic changes in the nuclei of the microspore mother cells in *Scævola Lobelia* Linn.

2. The leptotene threads are beaded with clear diamond-shaped areas. It is therefore probable that the threads are double.

3. There are eight diplotene pairs and these are of different lengths. The nature and number of the chiasmata in these pairs are followed.

4 The late diplotene chromosomes show the presence of two coiled strands in specially favourable preparations.

5 The haploid number of chromosomes is determined from several countings to be eight; this number also corresponds to the number of diplotene pairs.

6 After the second division in the mother cells, the microspores are formed which show the typical tetrahedral arrangement

7 The pollen grains are binucleate at the time of liberation from the anther locules

In conclusion it is my pleasant duty to express my gratitude to Dr M O P Iyengar, Director, University Botany Laboratory, Madras, for kindly suggesting this work Further, I am greatly indebted to Prof M A. Sampathkumaran for kind encouragement and helpful criticism during the course of this investigation

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A CONTRIBUTION TO THE EMBRYOLOGY OF THE VERBENACEÆ

BY KAILASH CHANDRA MISRA, M Sc

(From the Department of Botany, Benares Hindu University, Benares)

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THE present paper deals with the development of the flower, microsporangogenesis and the structure and development of the embryo-sac in *Clerodendron*, *Phlomis* Linn, *C. urticifolium* Wal, *C. macrosiphon* Hook, *Caryopteris Wallichiana* Schau, *Stachytarpheta indica* Vahl, *Gmelina arborea* Roxb, *Tectona grandis* Linn, *Lippia nodiflora* Rich, *Lantana Camara* Linn, and *Duranta Plumieri* Jacq. Out of these plants *Clerodendron*, *Phlomis* and *Caryopteris Wallichiana* have been studied in detail. Of the other plants only a small amount of material has been examined, as all the plants agree in their essential features.

The material of *Clerodendron urticifolium* and *C. macrosiphon* was brought by Dr. A. C. Joshi from the Royal Botanical Gardens, Sibpur, Calcutta. All the other plants were collected locally. The usual methods were followed in the investigation.

Previous Work

The work previous to 1930 has all been summarised by Schnarf (1931). A detailed review of this, therefore, is not necessary. The most important publications are those of Kanda (1920), Dahlgren (1923), and Schnarf (1923). All these investigations deal with the genus *Verbena*. Since then a very comprehensive work on this family has been done by Junell (1934). This is based on a study of a large number of genera and may be summarized as follows:

"Nucellus conforms to the typical sympetalous type. There is one integument. In *Avicennia* this does not cover the whole of the nucellus. The archesporium in the ovule is hypodermal in origin and may consist of one or more cells. Normally one of these cells develops into the megaspore-mother cell without cutting any parietal cell. The embryo-sac development corresponds to the *Normal*-type, but in the genus *Avicennia* perhaps it may agree with the *Scilla*-type. The shape of the mature embryo-sac is variable in different genera. The egg-apparatus consists of an egg-cell and two

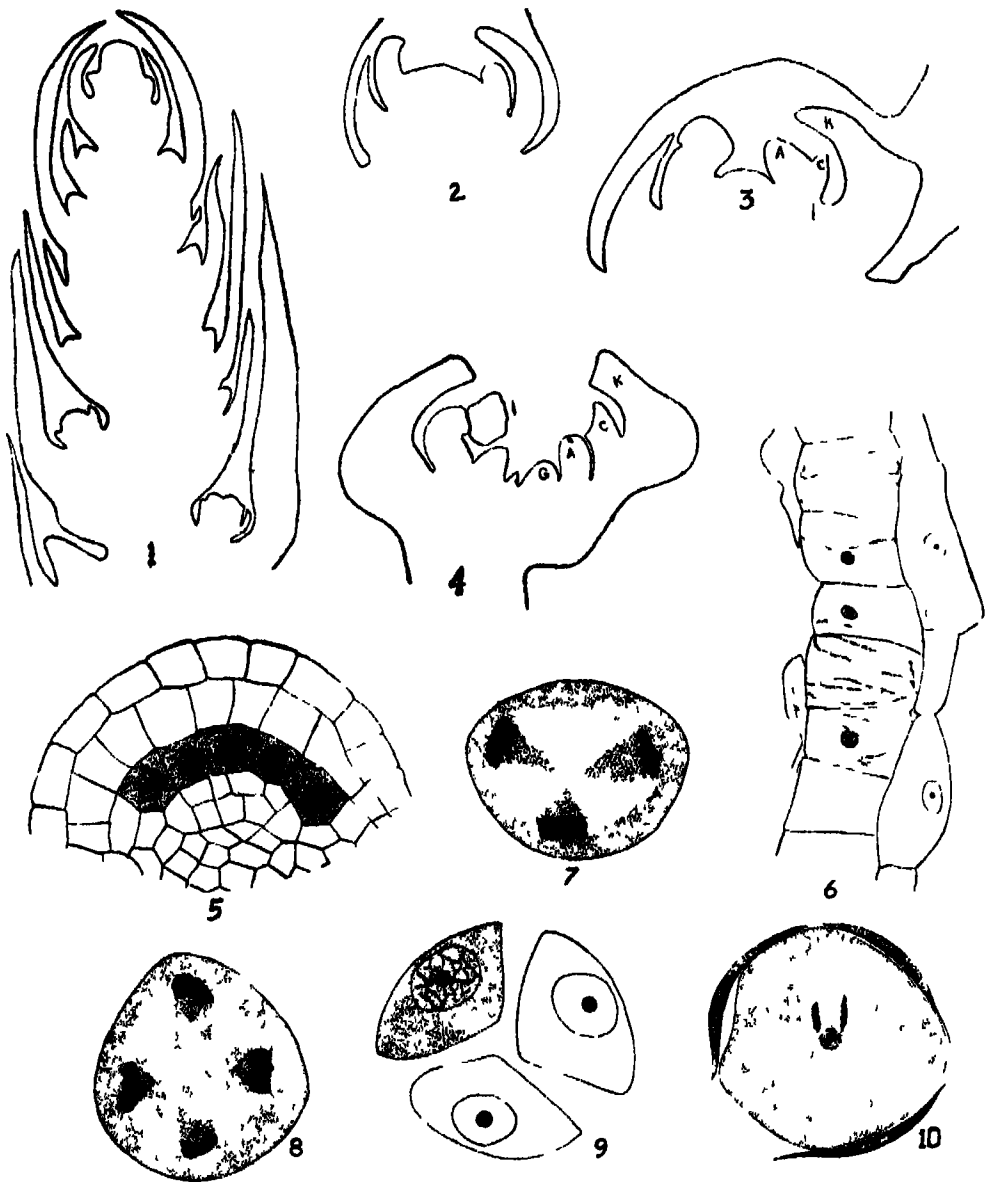
hooked synergids. In Verbenoideæ, *Pityrodia* and *Callicarpa* the polar nuclei do not fuse before fertilisation, but in *Clerodendron* and *Cornutia* they fuse before fertilization. The antipodals are three in the beginning, but they often divide afterwards and increase in number. They, however, never increase in size and generally degenerate early."

Organogeny of the Flower

The development of the various floral parts has been studied in detail in *Stachytarpheta indica*. It is seen that the appearance of a papilla in the axil of a bract (Fig. 1) is the first evidence of the formation of a flower. As the papilla develops, the calyx primordia appear at its base as an annular outgrowth (Fig. 1, lower end). Next to appear on the papilla are the combined stamen and petal primordia (Fig. 2). The stamens differentiate first out of the primordia and later the corolla separates off towards the outside (Fig. 3). The tip of the floral axis is utilised for the formation of the carpels (Fig. 4). As they develop, they become narrowed at apex and form the style and the stigma. The former is solid from the very beginning. During further development of the ovary the ovules appear as small protuberances from the ventral margins of the carpels. This account of differentiation of the various floral organs differs from that of Kanda (1920) in one respect. According to him the stamens appear first and then the sepals and the petals. In the present case the sepals are seen to appear before any other organs.

Microsporogenesis

The archesporium differentiates in each of the four lobes of the anther as a hypodermal layer, showing four to five cells in a transverse section and eight to nine cells in a longitudinal section of the anther. These cells by periclinal divisions form a layer of primary wall cells towards the outside and a layer of primary sporogenous cells towards the inside (Fig. 5). The former undergo further periclinal divisions to form the anther wall, which consists of four layers of cells including the epidermis. The differentiation of these layers agrees with that of *Rivina humilis* described by Joshi (1936). Out of the three layers developing from the primary wall cells, the innermost develops into the tapetum, the middle one is crushed during further development, while the outermost layer, that is the one just below the epidermis, forms the fibrous endothecium (Fig. 6). The tapetal cells are uni-nucleate in the beginning, but during the heterotypic and the homotypic divisions of the microspore-mother cells they become binucleate and much vacuolated. In this condition they remain for some time, but as the pollen grains are formed they degenerate. The tapetum is of the secretion type, and no periplasmodium-formation is observed.



FIGS 1-4 —*Stachytarpheta indica* Fig 1 longitudinal section of the apical portion of an inflorescence showing the differentiation of the floral rudiments $\times 48$ Figs 2 4, longitudinal sections of the flowers at different stages showing the differentiation of various floral parts $\times 125$

FIG 5.—*Clerodendron Phlomids* Transverse section of an anther lobe, showing the division of the archesporial cells into primary wall cells and the primary sporogenous cells $\times 445$

FIGS. 6-10 —*Caryopteris Wallachiana* Fig 6, portion of the mature anther wall, showing the epidermis and fibrous endothecium $\times 445$ Figs 7 and 8, two microsporocytes at the end of homotypic division $\times 1500$. Fig. 9, a tetrad of young, pollen grains $\times 1800$. Fig. 10, a mature pollen grain $\times 770$.

Simultaneously with the differentiation proceeding in the anther-wall, the microspore-mother cells complete the heterotypic and the homotypic divisions in the usual manner. Cytokinesis takes place by furrowing. The cleavage furrows appear at the periphery of the microspore-mother cells and extend up to the centre to separate the four pollen grains. The young pollen grains are mostly arranged in a tetrahedral fashion (Fig 9), but bilateral arrangement is also met with (Fig 8). The pollen grains are spherical except in *Stachytarpheta* where they are prominently three-lobed. These observations agree with those of Junell (1934). At the time of shedding the pollen grains are 3-nucleate (Fig 10).

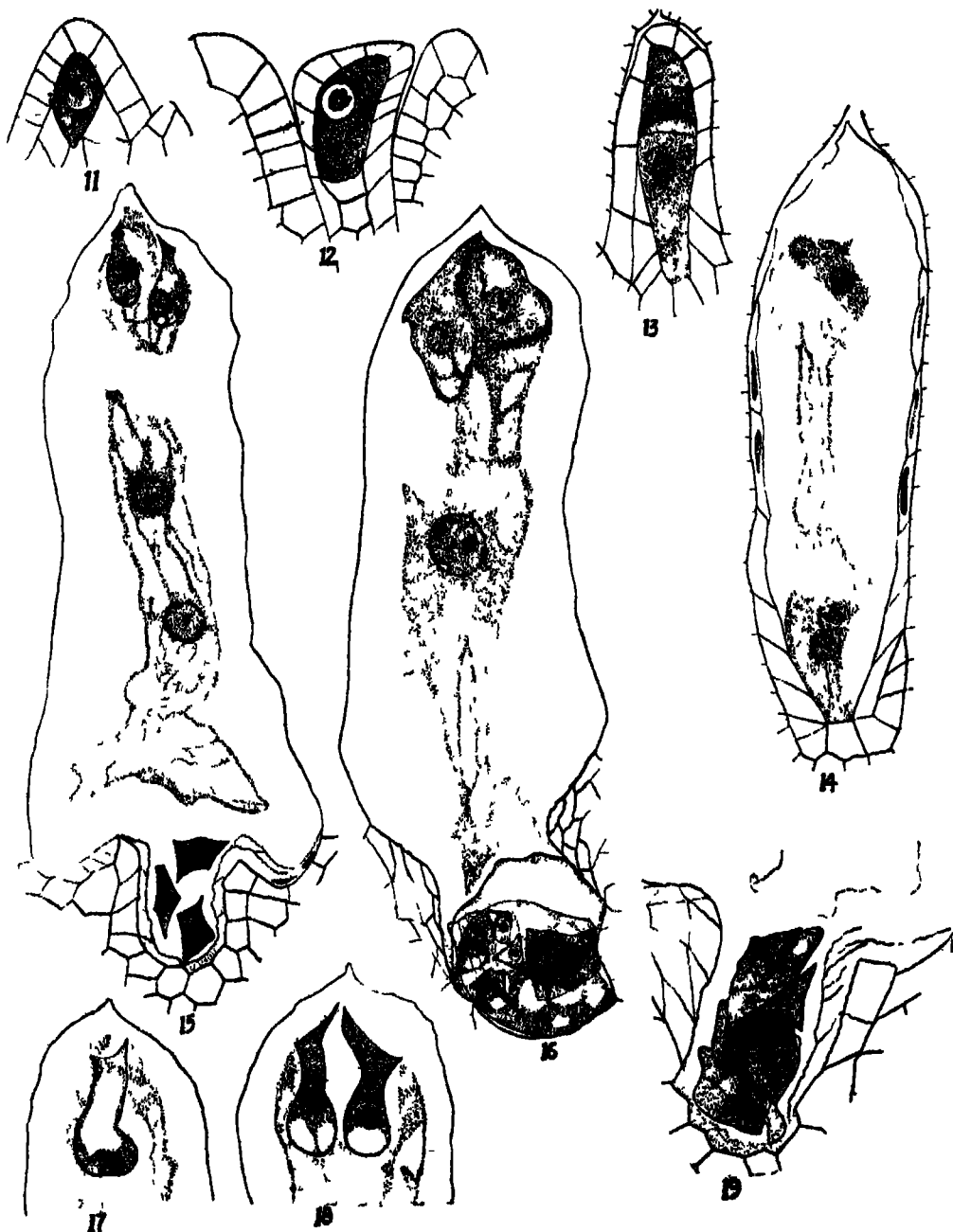
Megasporogenesis

The ovule is anatropous. In *Gmelina* it is attached to the placenta near the top of the ovary and is pendulous. In other cases it is attached between the base and the middle of the ovary.

The nucellus consists of a single layer of cells around the megaspore-mother cell. By the time the embryo-sac is formed it is mostly crushed. It thus agrees with the typical sympetalous type. The cells of the nucellus to begin with are all similar, but very soon a single hypodermal cell becomes larger and begins to take a deeper stain than the rest. This functions as the archesporium (Fig 11). The archesporial cell does not cut off any parietal cell, but directly functions as the megaspore-mother cell (Figs 12 and 20). This behaviour of the archesporium in the family has also been noted by all the previous workers. More than one archesporial cell has not been noted in any case, except perhaps in the example sketched in Fig 16.

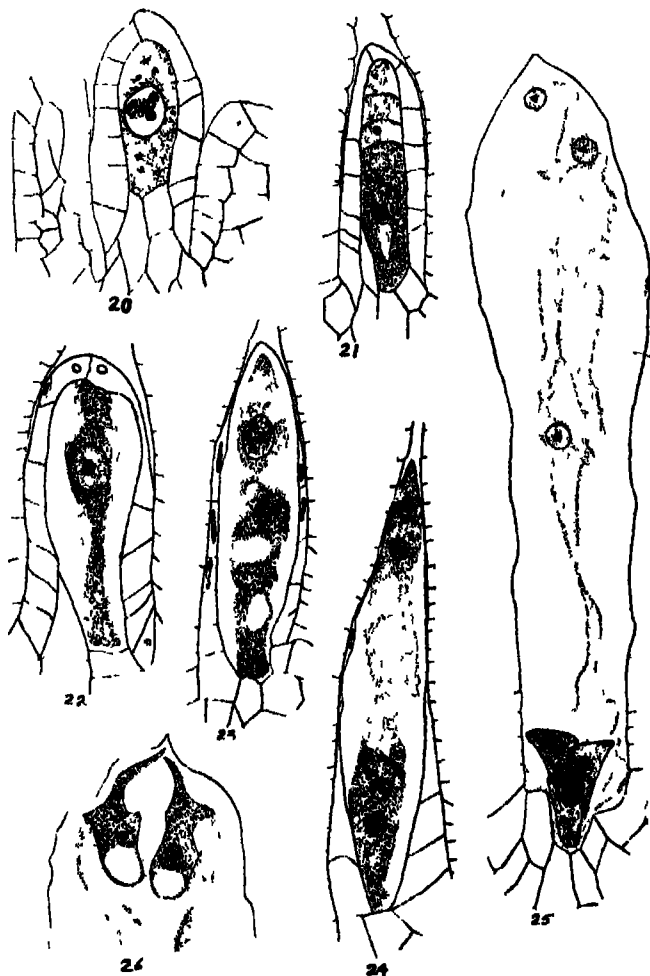
The megaspore-mother cell undergoes the heterotypic and the homotypic divisions and gives rise to a linear tetrad of megaspores (Figs 13 and 21). The integument makes its appearance as the archesporium differentiates, and by the time the four megaspores are formed the integument covers the entire nucellus (Figs 13 and 21). Out of the four spores of the linear tetrad, the three micropylar megaspores degenerate and the chalazal one develops into the embryo-sac according to the *Normal*-type (Figs 14-16 and 22-to 25). The antipodals are organised first and this is followed by the differentiation of the egg-apparatus.

The mature embryo-sac in species of *Clerodendron* is comparatively broad towards the chalazal end (Fig 15). In *Tectona*, *Stachytarpheta*, *Gmelina*, *Caryopteris* and *Duranta*, the embryo-sac is narrow towards the chalazal region and broad towards the micropylar side. In *Lippia*, the embryo-sac is tapering towards both the chalazal and the micropylar ends. In *Lantana*, the embryo-sac is comparatively small. It is narrow towards the chalazal end, but about one-third of the micropylar part is very broad.



FIGS. 11-19. *Clerodendron Phlomidis*. Fig. 11, an archesporial cell. Fig. 12, megaspore mother cell in synizesis. Fig. 13, linear tetrad of megaspores. Fig. 14, four-nucleate embryo-sac. Fig. 15, mature embryo-sac. One of the polar nuclei and the egg are not represented. Fig. 16, an example of an atypical embryo-sac. For further explanation see text. Fig. 17, the egg. Fig. 18, two hooked synergids. Fig. 19, chalazal part of the embryo-sac showing antipodals. Figs 11-18, $\times 445$, Fig. 19, $\times 770$.

The structure of the egg-apparatus agrees with the description given by Junell (1934). The egg is flask-shaped and shows a large vacuole towards the micropylar end (Fig. 17). The synergids are somewhat elongated pear-shaped structures and have a large vacuole in their chalazal region. The



FIGS. 20-26 *Cargopleris Wallachiana*. Fig. 20, a megaspore-mother cell in synizesis. Fig. 21, linear tetrad of megaspores. Fig. 22, 1-nucleate embryo-sac. Fig. 23, 2-nucleate embryo-sac. Fig. 24, 4-nucleate embryo-sac. Fig. 25, young 8-nucleate embryo-sac with the antipodals organised and the egg-apparatus not yet differentiated. One of the nuclei of the egg-apparatus and a polar nucleus is not seen in the figure. Fig. 26, two hooked synergids. $\times 145$.

nucleus is situated above the vacuole. They bear very prominent hooks and have very much prolonged acute beak-like apex (Figs. 18 and 26). One

case of an egg-like synergid was observed in *Clerodendron Phlomidis*. Here the nucleus was situated near the chalazal end and the vacuole above the nucleus and not below it (Fig. 15). Such egg-like synergids now seem to be of fairly common occurrence, as has been noted by Joshi and Kajale (1936).

The polar nuclei are comparatively larger than all the other nuclei of the embryo-sac. They fuse before fertilization. Sometimes starch grains are found associated with the secondary nucleus.

The antipodals in this family are described by Schnarf (1931) in his review of the embryology of the angiosperms to be three in number, small and degenerating early. Junell (1934), however, noted that the antipodals in this family frequently divide and their number increases. In the genus *Clerodendron* he has recorded the antipodals up to twelve in number. The writer has also noted the division of antipodals in *Clerodendron Phlomidis*. There are to begin with only three antipodals (Fig. 15), but these very soon undergo repeated divisions and their number increases up to twenty or a little more, as has been recently pointed out by the writer elsewhere (Misra, 1937). The antipodals are small in size and so are their nuclei, but they are very rich in cytoplasm (Fig. 19).

An unusual case from *Clerodendron Phlomidis* is sketched in Fig. 16. Here at the chalazal end of the embryo-sac, besides the normal antipodals, one unusually large cell is seen. It is uni-nucleate and much vacuolated. It is difficult to interpret its true nature. The cell in question can be either an exceptionally hypertrophied antipodal or an accessory uni-nucleate embryo-sac which may have developed from a second archesporial cell or from another megaspore of the same tetrad. The latter interpretation is supported by the fact that Junell (1934) has noted more than one sporogenous cell in some species of *Clerodendron*.

Summary

Organogeny of the flower and structure and development of the anther and embryo-sac have been studied in some members of the Verbenaceae. The development of the floral parts in *Stachytarpheta indica* shows that the calyx differentiates before the stamens and the petals, and not after the stamens as has been described by Kanda (1923) in *Verbena*. Development of the anther and the pollen is quite normal. Cytokinesis takes place by furrowing. Tapetum is of the secretion type. The pollen grains are mostly spherical, but in *Stachytarpheta* they are prominently three-lobed. The mature pollen grain is 3-nucleate. The ovule shows a single archesporial cell. No parietal cell is cut off and the archesporial cell directly develops into the megaspore-mother cell. A linear tetrad of megaspores is formed,

of which the chalazal megaspore develops into the embryo-sac according to the normal type. The egg-apparatus has a normal structure and so is the behaviour of the polar nuclei. The antipodals are small and three in number, but in *Clerodendron Phlomis* they multiply by repeated divisions to form about twenty cells. The nucellus is of the typical sympetalous-type.

Acknowledgements

In the end, I take this opportunity of thanking Dr A C Joshi, for his guidance and helpful suggestions throughout the progress of the investigation. I am also indebted to Dr Y Bhâradwâja for his kind interest in the work.

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ON THE NEPHRIDIA OF *PRIONOSPIO CIRRIFERA* WIREN

BY PROF R GOPALA AIYAR

(From the University Zoological Research Laboratory, Madras)

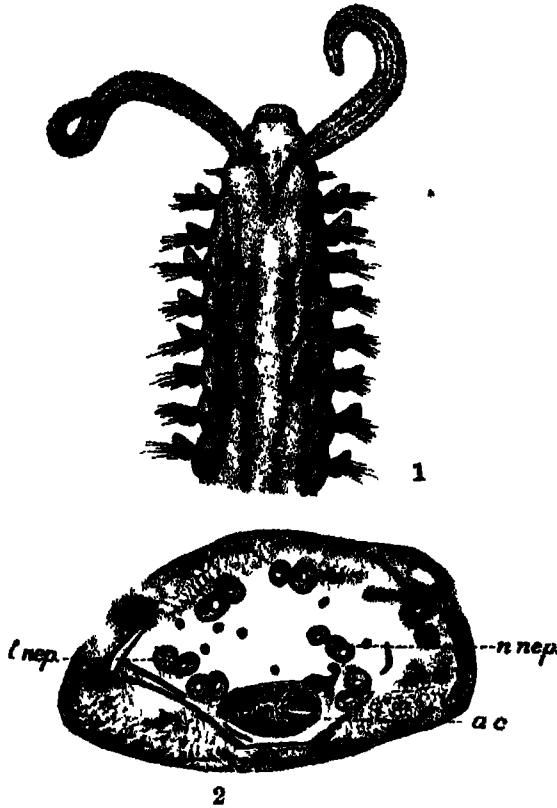
Received December 5, 1938

THIS spionid is very common in the brackish water, Madras, though Southern (1921) does not mention its occurrence in his *Polychæte fauna of the Chulka Lake*. Fauvel (1932) gives an account of this species in his *Annelida Polychæti of the Indian Museum*. He records it from Vizagapatam "bottom and channel". The worms are pinkish yellow generally with a green tinge at the anterior end. While examining the worms casually my attention was drawn to this beautiful green colour in front and after further examination under the binocular it was clear that the green colour is due not to any pigment in the body wall but to three pairs of long thread-like tubes occupying setigerous segments 4, 5 and 6. Each tube is in the form of a U with the two limbs closely pressed against each other in the anterior part and slightly twisted one over the other in the posterior. The course and the disposition of the tubes could be seen with the greatest clearness. Though the structures belong to segments 4, 5 and 6, they are so long that they can be seen to extend as far backwards as the ninth segment. This is rendered possible by the posterior septa belonging to segments 4, 5 and 6 being pushed back to the hind end of the pharynx where they find their inner attachment to the alimentary canal. They are kept in position by the presence of very slender muscle fibres passing between the hind ends of the nephridial loops on the one hand and the hind end of the pharynx on the other. Behind this region, from the twelfth segment onwards in mature worms, the segmental compartments are filled with reproductive elements, ova or sperms as the case may be. This reproductive region continues to the very posterior end of the worm. While the front part or the nephridial region is clear, enabling the nephridial tubes to be seen, the second part, the reproductive region, is opaque due to the closely packed reproductive elements.

Detailed Structure of the Nephridia

Microphotograph 1 and Text-Fig 1 show the nephridia as they appear in a living worm. For purposes of description the nephridium may be divided into four regions (1) the funnel, (2) the narrow straight tube leading backwards to (3) the U-shaped tube with two parallel limbs the

second of which leads into (4) the short terminal part opening to the outside by means of the nephridiopore (Microphotograph, 2) It will be seen that except at the two ends the limbs of the U-shaped structure are very close together. The internal opening is placed very near the body wall where the



TEXT-FIG. 1 —Diagram of the anterior end of the Polychaete showing the Nephridia shining through the body wall

TEXT-FIG. 2 —Transverse section of the worm showing the cut view of the 3 pairs of Nephridia.

anterior septum of the segment to which the nephridium belongs joins it. This opening is large and the lips which are strongly ciliated form a large clear funnel one side of which is prominent (Microphotograph 4) The walls of the funnel are formed of cells of large size with round nucleus and the lip by a single row of cubical cells. The nephridial funnel narrows and pierces the septum and then emerging into the posterior compartment merges into the anterior end of the nephridium proper which runs backwards having almost a straight course, then turns upon itself and runs forwards

as the distal limb in close contact with the proximal, then both the limbs turn sharply outwards, the two limbs now separate, the distal piercing the body wall and opening to the outside by the nephridiopore while the proximal, as already mentioned, pierces the septum in front and opens into the coelom by means of a funnel. The proximal limb of the nephridium is vivid green in colour, the second part is rather yellowish. The colouration in both cases is due to spherical granules of excreted matter. The lumen of the nephridium is clear and strongly ciliated (Microphotograph 4). The cilia in the funnel and in the terminal portions of the nephridium are very powerful while in the intervening region they are only of moderate length. Often, more particularly in the distal part, the granules may be noticed in the lumen of the organ. Suitable transverse and longitudinal sections reveal the fact that the entire nephridium is formed of a single row of cells fitting end to end like a string of pearls. The cells are large and to some extent vacuolated. The cell boundaries are perfectly clear. The lumen is central and the nucleus, which is almost spherical, lies close to the lumen on one side (Microphotograph 3 and Text Fig 2). There is a single strongly staining nucleolus. Often, almost the entire length of a limb of the nephridium gets cut longitudinally and it is then noticed that all the nuclei lie on one side of the lumen forming a definite row.

From the description given it will be obvious that the structures described are true nephridia of the drain pipe type opening at one end by means of the nephridiostome and at the other by a nephridiopore. In the region in which they occur no sexual elements are formed and consequently these structures cannot act as genital ducts. Besides, the lumen is intra-cellular which cannot admit the passage of eggs. The nephridia should be regarded as belonging to the nephromyxia type of Goodrich (1900). Now in regard to the nephridia of the genital region I am not able to throw any light. Careful sections have not revealed the presence of any nephridia similar to those described for some of the forms of the group, i.e., nephridia designed merely as passage for the reproductive elements. The genital elements could only get out by the rupture of the body wall.

In general shape the nephridia are very much like the nephridia of *Scalibregma inflatum* described by Ashworth (1902). Both have a U-shape, an internal opening by means of a funnel, and an external nephridiopore. There the agreement stops. *Scalibregma* may have as many as 40 pairs of nephridia while here there are three pairs only. Further in *Scalibregma* the nephridia have inter-cellular lumen while it is intra-cellular in *Prionospio cirrifera*.

Claparade and Metchnikof (1869) have described the segmental organ in a male *Spio Mecznihowianus* and mention the fact that the sperms could be seen inside the nephridia which occur in the middle and posterior segments

Cosmovice (1869-70) does not describe the segmental organ in spionids nor does Mayer (1886-87) in his *Körperbau der Anneliden*. Cunningham (1888) describes them in *Nerine cirratulus* and *N. foliosa*. They have large internal funnels, the coelomostomes, and distinct external openings, the nephridiopores. In *N. cirratulus* the structures act as genital ducts in both sexes while in *N. foliosa* sperms alone have been noticed to pass into the nephridia.

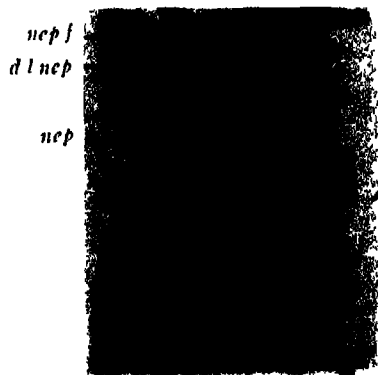
Cunningham and Ramage (1888) describe the nephridia of *Nerine foliosa* and *N. cirratulus* in the middle and the posterior regions. The nephridia are spherical with short internal and external ducts. They further state that there are no nephridia in the anterior part of the body. Species of *Polydora* are known to have well-developed nephridia with inter-cellular lumen in all the body segments except in a few of the very anterior and posterior segments.

Goodrich (1900) has stressed the mixed nature of the nephridia in spionids and pointed out that the internal funnel is coelomic while the conducting part is formed out of the excretory part. Fage (1905) brought out the same aspect of nephridial structure and mentions that in such forms like *Spio martinensis*, *Scololepis cihala* the segmental organs enlarge at the time of breeding so as to act as genital passages. The same point was stressed by Mesnil (1916) in his paper on the egg-laying habits of *Spio martinensis*.

Here in *Prionospio cirrifera* we have a type of organ described for the first time and very similar to that found in *Nereis* though not lodged in any compact tissue. It is not at all improbable that this condition has been secondarily acquired in this form. It is likely that this polychaete once possessed in the middle and the posterior segments nephridia of the nephromyxia type, that for some reason there was a suppression of these structures with a hypertrophy of the anterior nephridia. Complete atrophy of the body nephridia followed resulting in the genital products finding their way to the outside by the bursting of the body wall, a phenomenon which evidently occurs more frequently than is commonly realised.

Summary

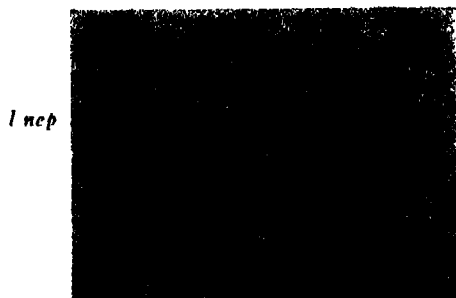
1. There are three pairs of U-shaped nephridia which occur in segments 4, 5 and 6.
2. A detailed description of the structure of the nephridia is given.
3. There are no nephridia in the middle and posterior segments. The genital products probably escape by rupture of the body wall.



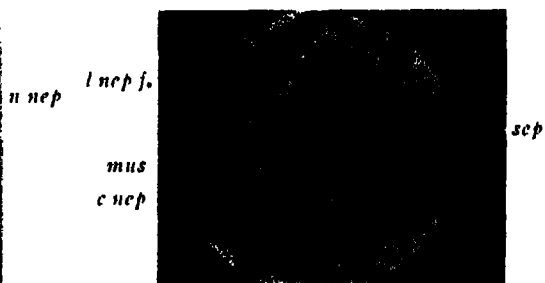
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EXPLANATION OF PLATE

FIG 1—Showing the three pairs of Nephridia of the living worm

FIG 2—An enlarged view of a portion of the worm showing the first portion of the proximal limb and the last portion of the distal limb of a Nephridium before the latter opens to the outside

FIG 3—To show the intra-cellular lumen of the Nephridia

FIG 4—Showing lip of funnel and the commencement of the Nephridium

KEY TO LETTERING

<i>a c.</i>	.	alimentary canal
<i>c.nep.</i>	.	cilia of nephridium.
<i>d.l.nep</i>	.	distal limb of nephridium
<i>l.nep.</i>	.	lumen of nephridium
<i>l.nep.f.</i>	..	lip of nephridial funnel
<i>mus</i>	.	muscle
<i>nep</i>	.	nephridium.
<i>nep f</i>	.	nephridial funnel
<i>n nep</i>	.	nucleus of nephridial cell.
<i>n p</i>	.	nephridiopore
<i>sep</i>	.	septum

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THE MYXOPHYCEÆ OF THE UNITED PROVINCES, INDIA—IV*

BY RAMA NAGINA SINGH, M Sc

Research Scholar in Botany, Benares Hindu University

Received November 22, 1938

[Communicated by Professor Y Bhâradwâja, M Sc, Ph D (London), F L S]

THE present communication deals with some of the Myxophyceæ that have not yet been recorded from the United Provinces. All the forms have been collected from the paddy-fields of Gorakhpur during the last two years. In all there are eleven forms, out of which, two species, two varieties and four forms are new.

SYSTEMATIC ENUMERATION OF THE SPECIES OBSERVED

I CHROOCOCCALES

Chroococcaceæ

Genus *Aphanothece* Nageli

1 *Aphanothece stagnina* (Spreng) A Braun Frémy, "Les Myxophycees de l'Afrique equatoriale francaise," *Arch d Bot*, iii, (1929), Mem 2, 1930, p 27, Fig 26, a and b

Lat cell, 4.5-6 μ , long cell., 7-9 μ

Habitat—Floating on the surface of water, along with young filaments of *Dichothrix*. (October 15, 1937)

II CHAMOSIPHONALES

Dermocarpaceæ

Genus *Stichosiphon* Gertler

2. *Stichosiphon indica* Rao Rao, "A new species of *Stichosiphon* (*Stichosiphon indica* sp. nov), *Proc. Ind Acad Sci.*, 1935, Vol II, p 536, Figs. 1-10.

Forma

Lat. cell., 4.2-6 μ , long cell, 15-20 μ , lat sporang, 6.6-7.4 μ , long sporang., 75-100 μ ; lat spor, 5-6.6 μ , long spor., 5-7.5 μ , lat spor spheric, 4-5 μ

* From the Department of Botany, Benares Hindu University.

Habitat —Epiphytic on *Cladophora* sp, growing on gastropod shells.
(Oct 30, 1937)

The form differs from the type in possessing bigger cells, narrower sporangia and smaller spores

III HORMOGONEALES

(a) *Nostochopsis* Wood

Genus *Nostochopsis* Wood

3 *Nostochopsis lobatus* Wood Geitler, in Rabenhorst's *Kryptogamenflora von Europa*, XIV Band, Cyanophyceæ, 1930-32, pp 476 and 477, Figs 285 a and b, and 286

Lat basal trich, 4.5-6.6 μ , long cell, 5-7 μ , lat cell higher up, 3.3-4 μ , long cell higher up, 9.9-10.5 μ , lat lateral het, 6.6-9.5 μ , long lateral het, 7-11 μ , lat intercal het, 6.6-8 μ , long intercal het, 6.6-8 μ

Habitat —Floating on the surface of water near the edge of a field
(Oct 15, 1937)

This species has not yet been recorded from India

(b) *Rivulariaceæ*

Genus *Glæotrichia* Agardh

4 *Glæotrichia Ghosei* sp nov (Fig 1, A-C)

Thallus free-swimming, 4-5 cm in diameter, spherical or almost ellipsoidal, brown. Filaments with a thin, stratified and brown sheath, sometimes constricted at intervals. Trichomes with constrictions at the joints, ending in long hairs. Cells at the base of the trichome barrel-shaped, much shorter than broad or almost quadratic, higher up cylindrical, in the hair long cylindrical. Heterocysts single and spherical. Spores long ellipsoidal with a hyaline smooth outer wall.

Diam fil up to 25 μ , long fil up to 1,000 μ , diam trich, at the base, 9-11 μ , higher up, 3.5-6.6 μ , long cell, at the base, 3-7 μ , higher up, 5-16.5 μ , lat het, 9.9-13.3 μ , long het, 9.9-13.2 μ , lat spor cum vag, 23.2-25 μ , lat spor, 13.2-16.5 μ , long spor, 46.2-50 μ , crass vag, 4-6.6 μ

Habitat —Floating on the surface of water, along with *Cylindrospermum muscicola* Kutz (November 2, 1936)

The alga approaches *Glæotrichia natans* Rabenh. in the presence of a thin, sack-like folded and constricted sheath, the long hairs, and the spherical

heterocysts, but, it differs from the same in the broader cells, bigger heterocysts and narrower spores, which are always ellipsoidal. It further contrasts with the above species in the absence of an intercalary meristem and in the presence of a narrower sheath.

(c) *Nostocaceae*

Genus *Cylindrospermum* Kütz

5 *Cylindrospermum gorakhporensis* sp. nov. (Fig 1, D)

Trichomes blue-green, single, with deep constrictions at the joints. Cells cylindrical. Heterocysts almost ellipsoidal, one at each end of the trichome, spores ellipsoidal with rounded apex, sub-terminal at either end of the trichome, with a thick yellowish-brown outer membrane provided with delicate needle-shaped projections.

Lat trich, 3.5–5 μ , long cell, 7–16.5 μ , lat het, 6–7.8 μ , long het, 13.2–16.5 μ , lat spor, 13.2–20 μ , long spor, 26.4–33 μ , lat spor without sheath, 13.2–14 μ .

Habitat—On moist soil, along with young filaments of *Oscillatoria* (Nov 5, 1937).

The distinctive features in this alga are the constricted trichomes, cylindrical cells and single ellipsoidal spores, with a thick, yellowish-brown and papillose outer membrane with delicate needle-shaped projections. It comes close to *Cylindrospermum minus* Kütz. on account of the ellipsoidal spores with papillose outer membrane and also in the breadth of the trichome, but, it differs from the same in the constricted trichomes, long cylindrical cells, bigger ellipsoidal heterocysts, and in possessing delicate needle-shaped projections in the outer membrane of the spore-wall. In the presence of cylindrical cells, constricted trichomes and spores with papillose outer membrane, the present alga agrees with *Cylindrospermum tropicum* W. et G. S. West, but, it contrasts with this species in bigger cells and heterocysts and smaller ellipsoidal spores with delicate needle-shaped projections in the outer membrane of its wall. The ellipsoidal heterocysts and spores with numerous delicate needle-shaped projections in the outer membrane of the spore-wall, characteristic of this alga also recall *Cylindrospermum trichospermum* Frémy, but it differs from the same in cylindrical and narrower cells with deep constrictions at the cross-walls, smaller heterocysts and much smaller spores with papillose outer membrane of its wall.

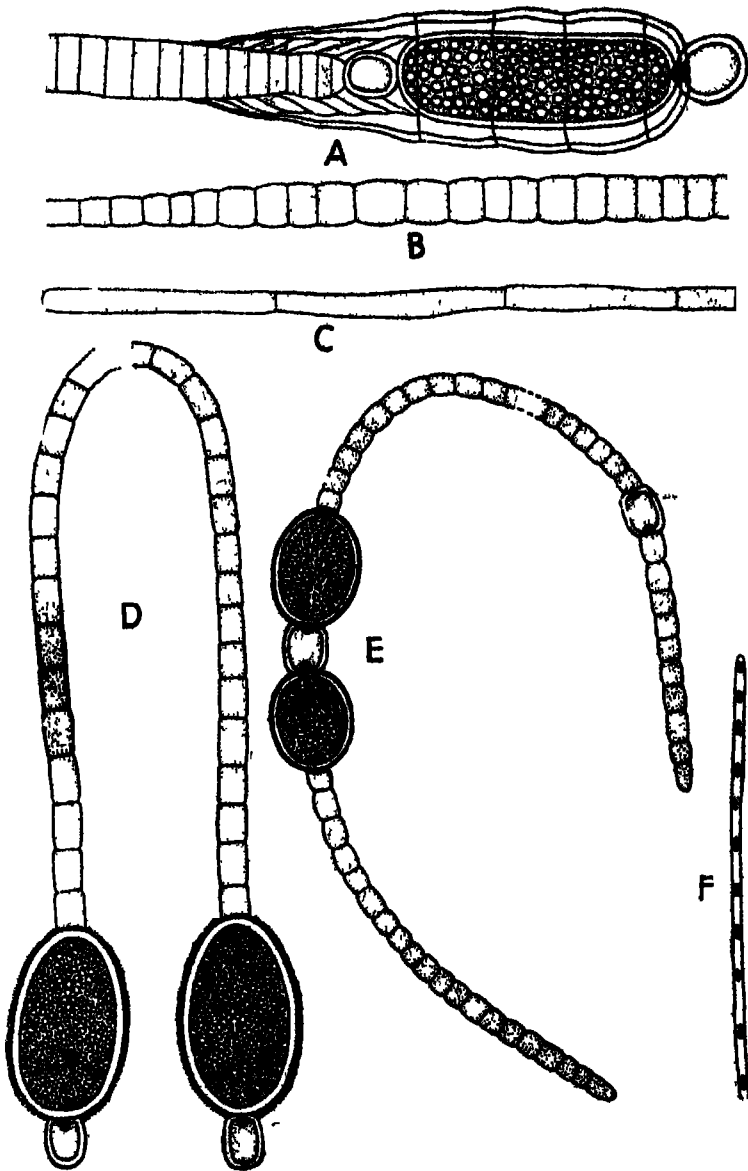


FIG. 1

A-lower, *B*-middle, and *C*-terminal portions of the filament of *Glasotrichia Ghosei* sp. nov., *D*-entire filament of *Cylindrospermum gorakhporensis* sp. nov., *E*-entire filament of *Anabaena lyngbyi* var. *unisporea* var. nov.; *F*-terminal portion of the filament of *Oscillatoria quadripunctulata* var. *ungranulata* var. nov.

A-E $\times 588$; *F* $\times 684$.

Genus *Anabæna* Bory

6 *Anabæna Iyengari* Bhâradwâja Bhâradwâja, "The Myxophyceæ of the United Provinces, India—I," *Proc Ind Acad Sci*, Vol 2, No 1, Sec B, 1935, Fig 6, H-K

Forma

Lat trich, 4.8-6.2 μ , long cell, 3.4-4.5 μ , lat het, 6.6-7.4 μ , long. het., 8-11 μ , lat spor, 13.2-14 μ , long spor, 15.20 μ

Habitat —Floating on the surface of water, along with sterile filaments of *Spirogyra* (October 15, 1936)

The form differs from the type in possessing broader spores, which are situated in pairs on either side of the heterocyst

7 *Anabæna Iyengari* Bhâradwâja Bhâradwâja, *op cit*, 1935, Fig 6, H-K

var *unispora* var nov (Fig 1, E)

Plant-mass mucilaginous, deep blue-green Trichomes single, free-swimming, straight, with conical apical cell, possessing almost rounded apex Cells barrel-shaped or almost quadratic Heterocysts barrel-shaped, rarely spherical Spores ellipsoidal or sub-spherical, one on either side of the heterocyst, with smooth and rust coloured inner wall

Lat trich, 3.3-4.2 μ , long cell, 3.3-5 μ , lat het, 4.5-6.6 μ , long het, 6.6-8.2 μ , lat spor, 13.2-16.5 μ , long spor, 16.5-20 μ

Habitat —Floating on the surface of water (November 2, 1937)

The variety resembles the type in conical end-cells, barrel-shaped cells and heterocysts adjoining the ellipsoidal spores, but, it contrasts with the same in possessing narrower cells and spores, which may be sub-spherical, placed singly on either side of a heterocyst

(d) *Oscillatoriaceæ*

Genus *Spirulina* Turp

8 *Spirulina spirulinoides* (Ghose) Geitler Geitler, *op cit*, 1932, p 922, Fig 590 (a)

Forma tenuis form nov

Lat trich, 2.3-3 μ , long cell, 8-11 μ , lat spir, 6-7 μ , spirals touching one another at 12-14 μ

Habitat —On moist soil, along with filaments of *Oscillatoria* (November 2, 1937)

The form differs from the type in possessing much narrower trichomes

Genus *Oscillatoria* Vaucher

9 *Oscillatoria proboscida* Gom Gertler, *op cit*, 1932, p 943,
Fig 598 b

Lat trich, 13.2-14.5 μ , long cell, 3.3-4.2 μ

Habitat —In muddy-waters, along with *Oscillatoria quadripunctulata*
var *unigranulata* var nov (October 20, 1936)

10 *Oscillatoria quadripunctulata* Brühl et Biswas, "The algæ of the
Bengal Filterbeds," *Journ of the Dept of Sci, Calcutta Univ*, 1922, Pl I,
Fig 6

var *unigranulata* var nov (Fig 1, F)

Lat trich, 1-1.7 μ , long cell, 7-8 μ

Habitat —In muddy-waters, along with *Oscillatoria proboscida* Gom
(Oct 20, 1936)

The alga differs from the type in possessing longer cells and also a single
granule on either side of the cross-wall

Genus *Microcoleus* Desmazieres

11 *Microcoleus subtorulosus* (Breb) Gom Gertler, *op. cit*, 1932,
p 1144, Fig 751

Forma

Diam fil, 13.2-20 μ , crass vag, 8-39.6 μ , diam trich, 6.6-9.9 μ ,
long cell, 4-5.5 μ

Habitat —Floating on the surface of water, along with young filaments
of *Nostoc* (October 20, 1936)

The form differs from the type in possessing shorter cells

In conclusion, I have much pleasure in expressing my great indebtedness
to Professor Y Bhāradwāja, for his kind guidance and criticism throughout
the course of this investigation

A CONTRIBUTION TO OUR KNOWLEDGE OF THE INDIAN SOIL ACTINOMYCETES

BY JAGJIVAN SINGH, M Sc , PH D (London)

Government College, Lahore

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[Communicated by Dr S L Ghose, M Sc , PH D (Cambridge), F R S]

1 Introduction

ACTINOMYCETES in the soil form a large and important group and their study has been of very recent origin. In India much work has not been done on these organisms except for some isolated attempts here and there. This work was started to get some information on the following points with regard to the behaviour of Actinomycetes in the Punjab soils.

I The seasons in the Punjab as already pointed out in another communication on soil bacteria (Singh, 1937), range from a very hot climate in summer to a very cold one in winter. It was considered interesting to know as to how these wide variations affect the Actinomycetes present in the soil throughout the whole year.

II How do the organic manures affect the Actinomycetes and how do their numbers compare with those reported by workers in other countries?

2 Previous Work

The study of Actinomycetes began with Globig (1888) who isolated a few thermophilic Actinomycetes. This was followed by Rossi-Doria (1891), Rullman (1895), Beijerinck (1900), Nadson (1903), Hiltner and Stormer (1903), Petri (1903), Gilbert (1904), Conn (1917), Waksman and Curtis (1916, 1919). All these workers confined themselves to isolating the different types of Actinomycetes.

Jensen (1930), and Subrahmanyam (1930) from the study of these organisms concluded that they occur in the soil only as conidia and not as vegetative mycelium. Subrahmanyam further obtained numbers of the order of one to three millions per gram in some Indian soils.

Fousek (1912) found that there is a greater proportionate increase of Actinomycetes (27 to 35 per cent) in fall than in spring (18 to 23 per cent), but there is no reduction in the winter. Hiltner and Stormer (1903) have observed an increase in the numbers of Actinomycetes in the autumn, and further they were found to form in spring 20 per cent, in the fall 30 per cent, dropping in winter to 13 per cent of the total soil microbial flora developing

on the plate Lockhead (*Soil and the Microbe*, p 65) could not trace any marked fluctuations of the Actinomycetes numbers in the different seasons of the year

Singh (1937) at Rothamsted could find no definite relation between the numbers of Actinomycetes and seasonal changes

3 Experimental Work and Technique

In a previous communication by the author (1937) experimental work and technique with regard to bacteria has been described and the same dilutions as for bacteria were used for this work. Nine plates in parallel were used for each plot, and incubated at 20°-25° C. The media used was Conn's asparaginate agar with pH 7.2. The counts on the plates were made on the Seventh and the Ninth day.

4 Results

Date	Plot	Moisture per cent	Average colony	Standard error
14th Dec 1935	Manured	17.88	35.0	± 2.14
	Unmanured	15.00	26.8	± 2.30
25th Dec 1935	Manured	20.48	33.5	± 2.63
	Unmanured	18.00	15.5	± 2.35
11th Jan 1936	Manured	18.56	45.5	± 1.73
	Unmanured	16.38	36.0	± 1.40
25th Jan. 1936	Manured	18.10	44.81	± 1.83
	Unmanured	15.66	41.62	± 2.30
8th Feb 1936	Manured	21.96	41.0	± 1.73
	Unmanured	21.54	33.9	± 2.60
23rd Feb 1936	Manured	19.46	38.1	± 1.68
	Unmanured	18.12	25.6	± 1.20
7th March 1936	Manured	16.68	36.6	± 0.90
	Unmanured	15.80	30.4	± 1.37
21st March 1936	Manured	21.28	37.7	± 2.00
	Unmanured	20.50	47.5	± 1.51
6th April 1936	Manured	23.04	43.9	± 1.50
	Unmanured	21.14	46.9	± 2.20
18th April 1936	Manured	15.80	38.7	± 2.20
	Unmanured	15.38	37.8	± 1.00
2nd May 1936	Manured	18.92	44.8	± 1.76
	Unmanured	16.04	30.3	± 2.73
16th May 1936	Manured	13.84	23.7	± 0.94
	Unmanured	13.64	23.0	± 1.70
30th May 1936	Manured	8.20	31.1	± 1.41
	Unmanured	5.00	26.9	± 0.80

Date	Plot	Moisture per cent	Average colony	Standard error
13th June 1936	Manured	16.52	23.0	± 1.80
	Unmanured	15.06	26.7	± 1.43
27th June 1936	Manured	17.40	35.1	± 1.15
	Unmanured	14.16	43.1	± 2.50
11th July 1936	Manured	19.52	26.2	± 0.90
	Unmanured	17.12	26.0	± 1.00
26th July 1936	Manured	13.24	33.5	± 1.85
	Unmanured	11.16	33.0	± 2.10
8th Aug. 1936	Manured	10.32	27.7	± 2.40
	Unmanured	8.40	25.8	± 1.85
22nd Aug. 1936	Manured	22.00	30.0	± 1.85
	Unmanured	17.76	26.7	± 1.43
5th Sept. 1936	Manured	15.50	21.3	± 1.29
	Unmanured	14.00	17.3	± 0.83
19th Sept. 1936	Manured	17.04	24.6	± 0.36
	Unmanured	16.62	19.5	± 0.50
3rd Oct. 1936	Manured	18.80	30.0	± 0.91
	Unmanured	17.54	33.4	± 1.32
17th Oct. 1936	Manured	17.36	31.4	± 0.90
	Unmanured	16.92	29.7	± 1.06
1st Nov. 1936	Manured	22.24	26.2	± 1.06
	Unmanured	20.20	21.1	± 0.80
14th Nov. 1936	Manured	22.72	24.4	± 0.91
	Unmanured	19.04	20.4	± 1.37
28th Nov. 1936	Manured	16.20	26.6	± 1.80
	Unmanured	15.80	18.6	± 0.84

Discussion

It has already been pointed out that this work was undertaken to get more or less a definite answer to the question as to how the different marked seasonal variations affect the intensity of the Actinomycetes in the soil. Punjab is one of the few places in the world where the exact behaviour of the Actinomycetes can be known with regard to seasonal changes. Hiltner and Störmer (1903) and Fousek (1912) have reported some proportionate increases in autumn and spring of the numbers of Actinomycetes, while Lockhead (1924) and Singh (1937) were unable to trace any marked changes in the numbers during the different seasons of the year. It is quite evident that the numbers would fluctuate from one sampling to another throughout the whole year but these fluctuations seem to scatter about a mean value.

and that the maximum in spring, for example in the manured plot in Fig 1, is about the same, as the maximum point in the mid-winter, i.e.,

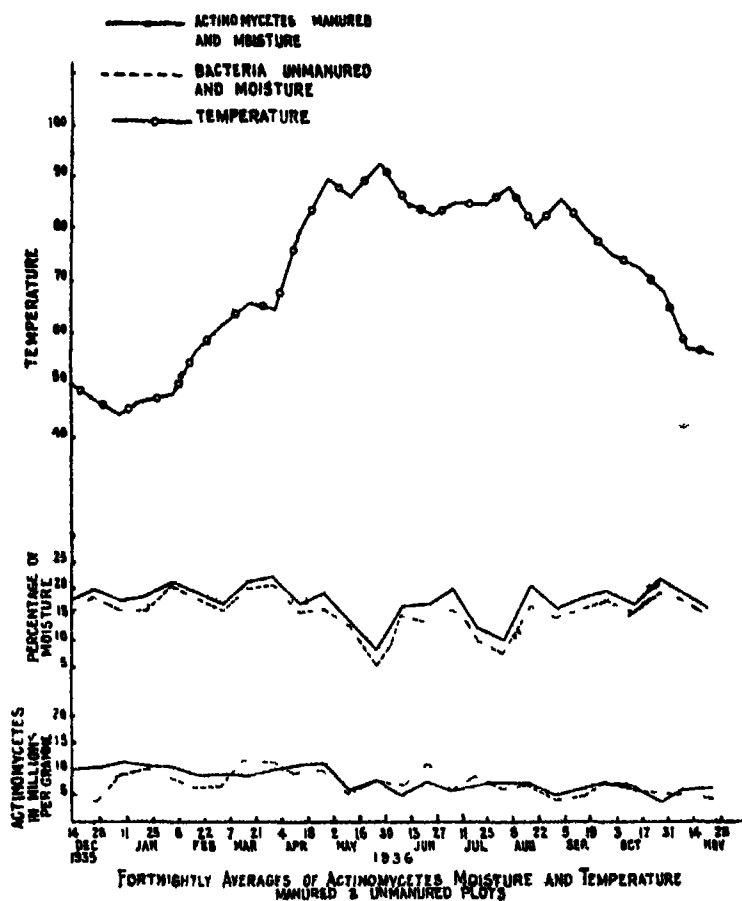


FIG 1

January Similarly in mid-summer and autumn the highest points are near about the same value. What is true of the manured plot holds good also for the unmanured plot. The evidence on the whole seems to indicate that Actinomycetes in the soil are not markedly affected by even the extremes of climatic variations, although one might perhaps have expected that. It can safely be said now that this group of soil organisms can be placed in a group in themselves—a group which on account of its inherent tenacity is not prone to any marked changes in the populations by the different conditions outside.

Summary and Conclusions

It can be said that Actinomycetes do not show any periodic phenomenon such as we find in the other groups of soil organisms. The curve on Fig 1 shows that the numbers of Actinomycetes seem to scatter about a mean value between four to eleven millions per gram of dry soil.

In the Punjab soils the numbers of Actinomycetes is slightly greater than those reported by the American and European workers.

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STUDIES IN THE ORDER PARIETALES

II. A Contribution to the Morphology of *Garcinia livingstonii* T. Anders

BY V PURI, M.Sc.

Meerut College, Meerut

Received November 28, 1938

(Communicated by Dr P Maheshwari, D.Sc.)

IN 1911 Treub reported that the development of the embryo-sac in *Garcinia kydia* and *G. treubii* proceeds normally up to the 4-nucleate stage but that thereafter only one of the micropylar nuclei divides further, while the remaining micropylar nucleus and the two chalazal nuclei do not divide at all. The resulting embryo-sac is thus 5-nucleate. Of the three nuclei at the micropylar end the two sister nuclei formed by the last division form the synergids and the third organises into the egg cell. The two chalazal nuclei are said to move up to the centre of the embryo sac and fuse to form the secondary nucleus. Following Treub, Rutgers (1923) also described an exactly similar type of development for *Moringa oleifera*. A re-investigation of this species revealed, however, that the interpretations of Rutgers were incorrect (Puri, 1934). The embryo-sac here is formed normally but, due to the early degeneration of the antipodal cells, it later *appears* to be only 5-nucleate.

This solution of the embryo-sac of *Moringa* prompted me to undertake a re-investigation of *Garcinia* as well. Material of the two species investigated by Treub being unfortunately unobtainable, my observations have been made on *Garcinia livingstonii*, collected from the Government Botanical Gardens, Saharanpur.¹

The Flower

The flowers are borne in clusters and appear to be peeping out of the surface of older branches without having any apparent connection with the leaves (Fig 1). (Such cauliflory is a phenomenon of frequent occurrence in woody plants of the tropical forests). Each flower possesses a long pedicel and two or three bract-like sepals which show a great variation in size but seldom exceed 1/3 of the size of the petals in open flowers. The latter are five in number and have a yellowish-green colour at the time of opening. Within these there are about 15 staminodes which arise rather irregularly from the base of the disc and reach from 1/4 to 1/3 the length of the ovary.

¹ I am grateful to Mr. F. O. Fordham, Superintendent of the Gardens, for kind permission to collect this material and sending some fixed material later by post.

I could never find any mature pollen grains in them. The last stage which I came across, and that too only in a few cases, was that showing immature, one-nucleate degenerating pollen grains. Engler and Prantl (1925) mention the occurrence of both uni-sexual as well as hermaphrodite flowers in the section *Teracentrum* to which *Garcinia livingstonii* belongs. In my collection, however, I did not find any really hermaphrodite flowers.



FIG 1 (× 18)

Vertical section through a portion of stem bark showing the emergence of three young buds.

The ovary is borne on a prominent disc. It is syncarpous, consisting of usually two and sometimes three carpels with the corresponding number of loculi, each with one ovule. Above the insertion of the latter the loculi usually become continuous through the disappearance of the septum (Fig. 2, *a*, *b*). The placentation is axile. In younger stages the carpels are fused below (Fig. 2, *d*) but free in the styler region (Fig. 2, *e*). In older stages, however, they fuse together completely (Fig. 2, *n*). The stigma is almost sessile and covers the top of the ovary.

The ovary wall is very thick and is at first composed of homogeneous cells. Later on, the region lying outside the vascular bundles becomes conspicuous by the development of numerous secretory canals which become smaller and smaller toward the periphery (Fig. 2, *b*). The cells in the outer region take up a darker stain on account of some tannin-like contents.

Vascular Supply—A ring of vascular bundles enters the pedicel of the flower. In the receptacular region one trace is given out to each sepal which

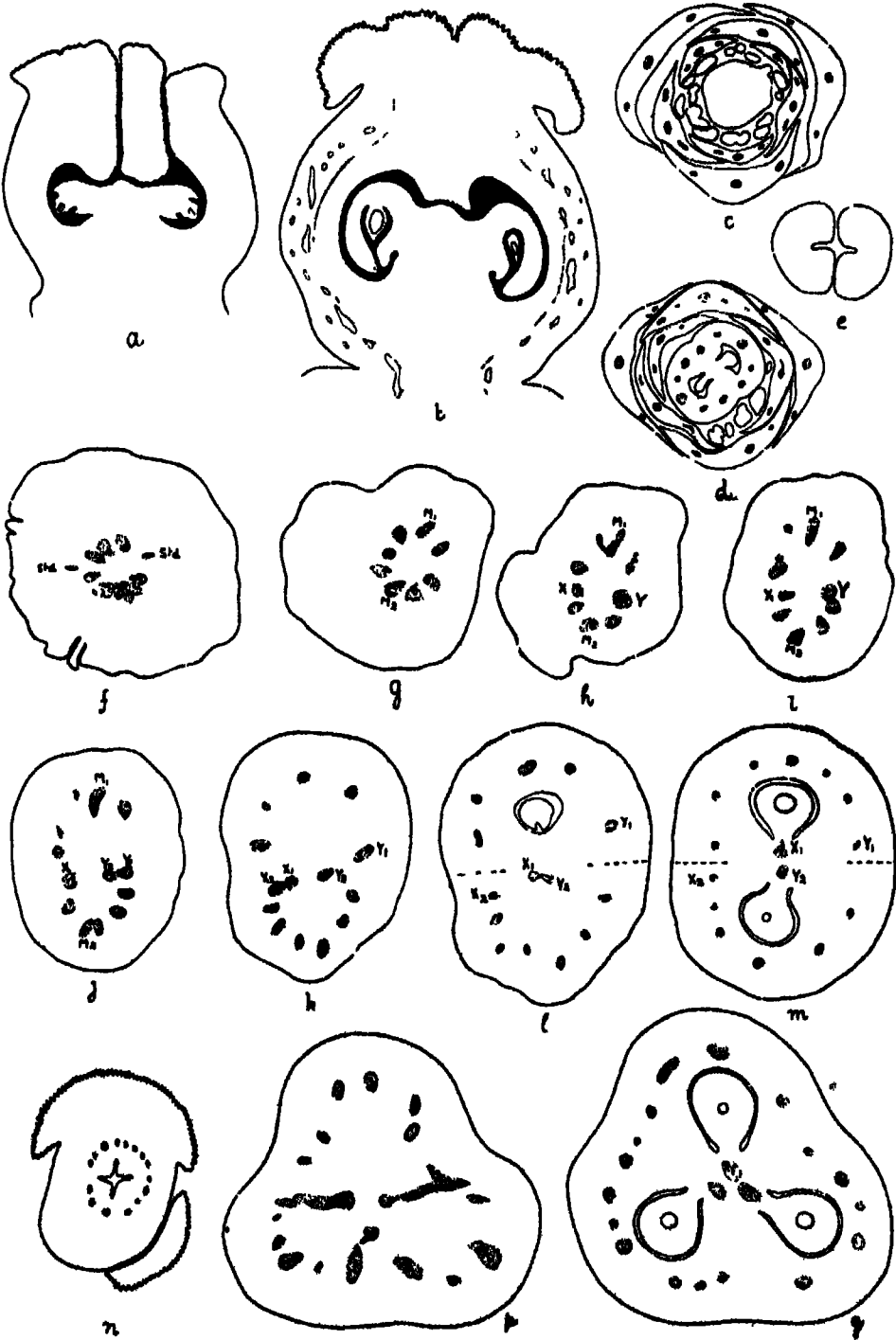


FIG. 2, a-q

Fig 2 a—Vertical section of a young ovary showing the orientation of the ovules ($\times 20$) b—Vertical section of a mature ovary showing the anatropous ovules ($\times 20$) c—Transverse section of a young bud ($\times 20$) d—The same but at a higher level ($\times 20$) e—T's through the styler region of a young ovary ($\times 20$) f-n—T sections passing through different regions of a bicarpellary ovary from below upward ($\times 18$) p-q T sections through a tricarpellary ovary ($\times 18$)

splits up, during its course, into three bundles—a mid-rib and two laterals. The petals also receive one trace each, which behaves in a similar fashion. The receptacular stele is provided with so many bundles that the passing out of sepal and petal traces does not affect it to any appreciable extent.

As the region of the disc is approached, small traces begin to pass out almost horizontally to enter the staminodes which arise from its base. Each staminode receives a single trace which runs unbranched throughout its entire course. As these traces pass out they leave behind a small amount of vascular tissue which soon disappears in the disc leaving behind somewhat deeply staining parenchymatous cells.

After the traces for the staminodes have passed out, there are still left usually eight and in some cases even more bundles in the receptacle. Now, the two bundles lying opposite to each other (M_1 and M_2) begin to move out to become mid-rib bundles of the two carpels (Fig 2, h). They are immediately followed by bundles lying on either side of them, all of which may undergo some secondary branching and anastomoses (Fig 2, i-l). There are, however, two bundles (X and Y) which remain in their original positions and their behaviour presents some interesting features. Each of them spreads out toward the centre and becomes tangentially constricted into an outer and an inner bundle (Fig 2, h, X_1 , X_2 , and Y_1 , Y_2). The bundles X_2 and Y_1 , which are in the peripheral region, move out in such a way that they come to lie in the margins of two different carpels (Fig 2, m). The other bundles, X_1 and Y_2 , meet in the centre, although still retaining their individuality, and each of them passes out into one ovule. X_1 supplies the ovule of the carpel receiving Y_1 and Y_2 enters the ovule of the carpel receiving X_2 . Thus the sister bundles X_1 , X_2 and similarly Y_1 , Y_2 enter into different carpels, one receiving X_1 and Y_1 and the remaining two passing into the other. The significance of this point will be dealt with in discussion.

The Ovule

The young ovule makes its appearance as a small protuberance consisting of homogeneous cells. At a very early stage, as a result of a quicker growth of the cells on one side, it begins to bend over to the opposite side. By further growth in the same direction it comes to lie along the longitudinal axis of the ovary with the micropylar end directed downward (Fig 2, a-b).

Usually a single hypodermal archesporial cell becomes differentiated (Fig 3, *a*) Sometimes two or three cells become prominent but it is only in exceptional cases that more than one develops further (Fig 3, *c*)

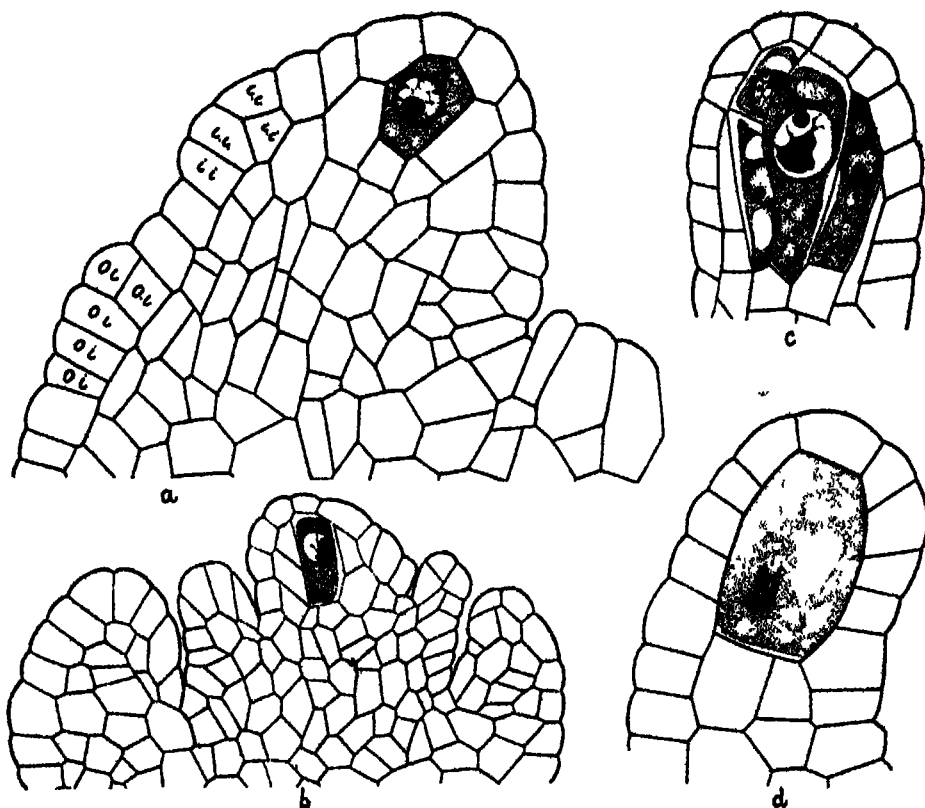


FIG 3, *a-d*

a—Vertical section of a very young ovule showing a single archesporial cell and the origin of the two integuments ($\times 700$) *b*—An older stage of the same ($\times 420$) *c*—Vertical section of a portion of aprellus showing two megaspore mother cells and a dyad ($\times 700$) *d*—Obliquely cut telophase spindle of the first division of the megaspore-mother cell ($\times 700$).

Both the integuments appear almost simultaneously at the time of differentiation of the archesporium. In the case of the inner integuments three epidermal cells elongate in the radial direction, and, to begin with, the uppermost divides periclinally (Fig 3, *a*). Later on the lower cells do the same and develop into a protuberance. The three layers in this integument are thus visible from the very beginning. The outer integument arises similarly but the number of cells taking part in its origin is larger (4-5 or even

more) and this results in the formation of a several layered tissue. Although arising at a lower level, it soon overtakes the inner integument and ultimately gets much ahead of this so that in the mature embryo-sac the long narrow micropyle is formed almost entirely by the outer integument (Figs 3, *b* and 2, *b*).

The archesporial cell does not cut off any parietal tissue but directly becomes the megaspore-mother cell. The latter enlarges considerably at the expense of the neighbouring cells. In Fig 3, *d* where the nucellus is comparatively narrow and pointed the mother cell occupies all the space beneath the nucellar epidermis, but this was the only example of this kind that I could find.

Tetrad Formation

Figs 3 *b, c* show the early prophase stages of the first division of the megaspore-mother cell. In Fig 3, *d* the telophase spindle is cut somewhat obliquely. This division is followed by a transverse partition resulting in a dyad. The lower of the two dyad cells is invariably the larger. They now undergo the second division and form the usual tetrad of four megaspores of which the chalazal alone functions (Fig 4 *a*).

Embryo-sac Formation

The first nuclear division in the functioning megaspore is followed by a separation of the daughter nuclei, one to each pole (Fig 4, *c*). Subsequent divisions result in the 4- and finally in 8-nucleate embryo-sacs (Figs 4, *c, d₁, d₂*).

Even at the four-nucleate stage, the whole of the nucellus including even the epidermis, becomes absorbed by the developing embryo-sac. Only a few nucellar cells are left on either side of the sac in the chalazal end. Subsequently they are also used up and the mature megagametophyte is directly bounded by the inner integument. In some cases even the latter is also attacked.

The Mature Embryo-sac

The mature embryo-sac has the usual organisation. It is spindle-shaped in outline, the two ends being very pointed and appearing to be haustorial in function (Fig. 4, *e*). The egg apparatus consists, as usual, of two synergids and an egg. Judging from the position of the nuclei of the micropylar quartet (Fig. 4, *d₁, d₂*) it seems that the synergids are sister cells. They are usually pyriform, elongated and have a vacuole at their lower ends (Fig. 4, *e*). In some cases they are provided with very prominent hooks

(Fig 4, f) The egg is the largest of the three cells and unlike the synergids it has its nucleus in the lower part and vacuole in the upper

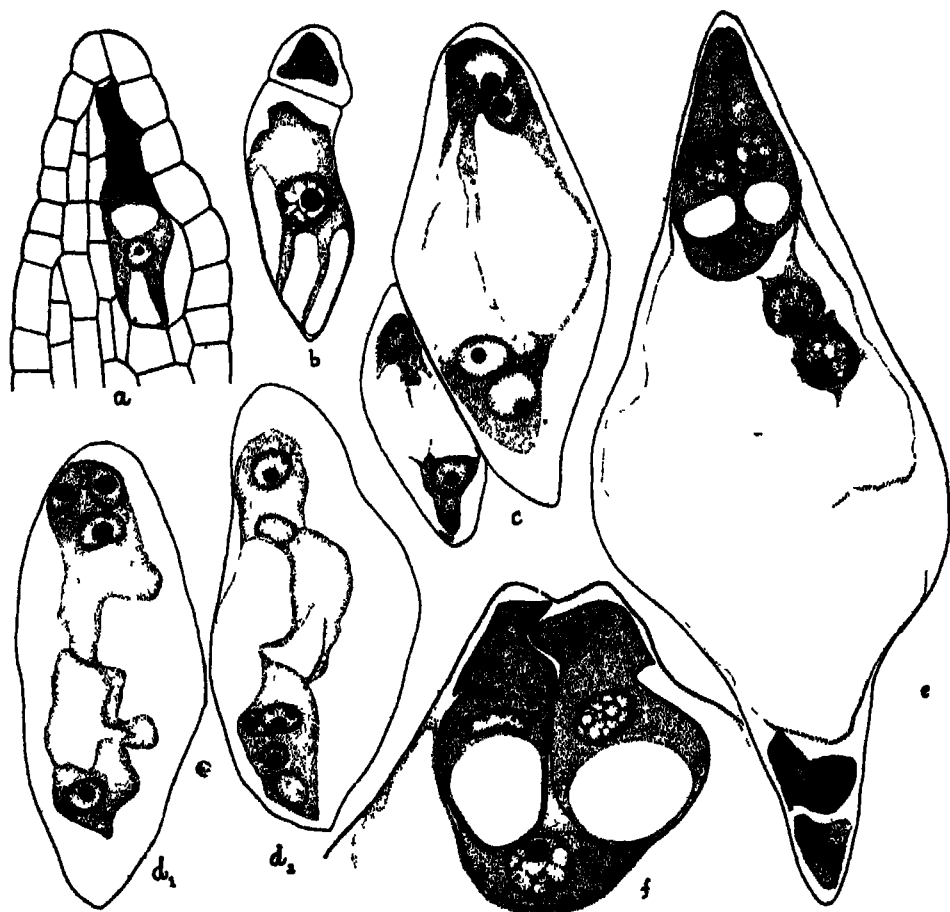


FIG 4, a-f ($\times 700$)

a Vertical section of the nucellus showing a tetrad of megaspores the upper three of which have degenerated b—Functioning megaspore c—Two embryo-sacs lying one above the other d_1 – d_4 —8-Nucleate embryo-sac e—Mature embryo-sac with degenerated antipodal cells (reconstructed) f—Abnormally large egg apparatus showing hooked synergids

The polar nuclei usually lie in the upper part of the embryo-sac just beneath the egg apparatus. The antipodal cells are very ephemeral, sometimes disappearing very early leaving only a 5-nucleate embryo-sac. It is probably this that misled Treub (1911) to imagine that only 5-nucleate embryo-sacs were formed in the species investigated by him. Sprecher (1919), however, reported normal 8-nucleate embryo-sacs in *Garcinia mangostana*.

5-Nucleate Embryo-sacs

The early disappearance of the antipodal cells has been responsible for a good deal of confusion in the literature on the development of some embryo-sacs in angiosperms. On several occasions it has misled authors to interpret a normal 8-nucleate embryo-sac as 5-nucleate (4-nucleate after polar fusion). I have already demonstrated that the embryo-sac of *Moringa oleifera* is 8-nucleate (Puri, 1934) although Rutgers (1923) reported a 5-nucleate condition. In a few species of *Carica*—*C. chrysopelala*, *C. pentagona*, *C. candamarcensis* and *C. papaya*—Heilborn (1921) described the usual condition as 5-nucleate but later on (Heilborn, 1928) he came across some 8-nucleate embryo-sacs also in his preparations. Agharkar and Banerji (1930) have shown clearly that in *Carica papaya* the mature embryo-sac is 8-nucleate. In *Sisyrinchium anceps* also Haeckel (1930) reported the 5-nucleate condition but since then she has been able to find the antipodals in her slides although they are very small and ephemeral (see Maheshwari, 1937, p. 365, foot-note 3). In *Eugenia jambos* and *Eugenia bancana* Pijl (1934) has described the same type of embryo-sac development as Treub (1911) gave for the two species of *Garcinia*. His account, however, is very incomplete and it seems quite probable that a re-investigation may show the normal 8-nucleate embryo-sac, so common in other members of the family. Similarly the 5-nucleate reports for *Cinnamomum camphora* (Giuliani, 1928), *Nipa fruticans* (Radermacher, 1925), *Chamaedorea concolor* (Suessenguth, 1921), *Antirrhinum majus*, *Linaria vulgaris*, *Melampyrum partense*, *M. silvaticum* and *Tozzia alpina* (Schmid, 1906)¹ appear to be doubtful and need verification. For *Linaria genestae-folia* Persidsky (1934) writes that the antipodals are "apparently" not formed at all.

Sometimes the 5-nucleate condition has been reported in exceptional cases as in *Myricaria germanica* (Frisendahl, 1912).

The genuine cases of 5-nucleate embryo-sacs are, however, very few. So far they have been reported in the *Podostemonaceae*, *Orchidaceae*, *Butomaceae*, and *Alismaceae* (see Maheshwari, 1937).

Discussion

Basal Disc—The basal disc in *Garcinia livingstonii* is a rather conspicuous structure. The nature and function of such a disc has been the subject of some controversy for many years. Kerner and Oliver (1902) appear to regard it as formed by metamorphosed leaves while Goebel (1905) speaks of it as a "luxuriant growth of the torus forming an annulus beneath the

¹ See Maheshwari, 1937, p. 365, foot-note 1.

ovary, functioning as a honey disc" Moore (1936) concludes that in the Phaseoleæ the disc is probably the modified outer whorl of stamens

In *Garcinia livingstonii* the study of the vascular supply does not seem to throw any light on the nature of the disc. Here it lies in between the staminodes and the ovary and thus appears to go against the view that it is formed by metamorphosed leaves especially the stamens³; for reduction and sterilisation in the andrœcium are generally met with in the outer whorls of stamens and not in the inner as the acceptance of the above view demands. In the Polemoniaceæ also the condition is similar but in spite of this Dawson (1936) regards it as representing "the enlarged fused bases of a whorl of stamens together with accessory parenchymatous tissue"

Nature of the Carpels —The larger number of bundles entering the base of the ovary in *Garcinia livingstonii* suggests that its immediate ancestors possessed at least four carpels a condition still exhibited in certain species of the genus. Later on, however, one or two carpels got eliminated but the vascular supply intended for them persisted and ultimately became distributed between the existing carpels. Such a condition, where the vascular supply of the eliminated carpels is made use of by the persisting ones, has been noted by other authors, as well. For instance, Chapman (1936) has found it in some of the Berberidaceæ and I have myself come across similar cases in some of the Capparidaceæ

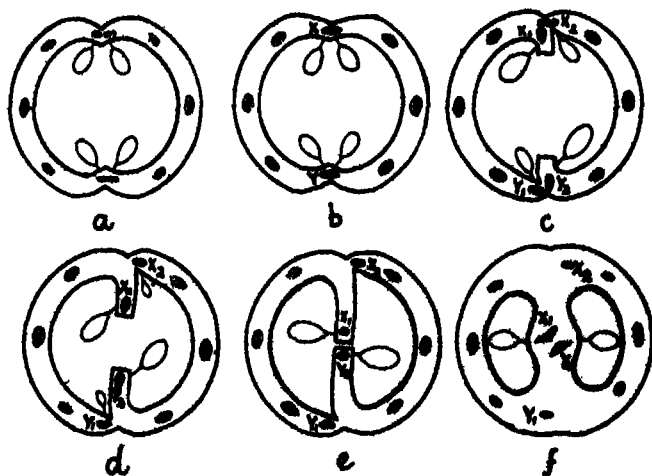


FIG. 5, a-f

Diagrams illustrating the interpretation of the ovary proposed here.

³ I have no evidence to suggest that it represents modified carpelodes.

Fig 5, *a, b* attempt to show the condition which might have been in existence when the bi-carpellary condition first appeared. At this stage the ovules were borne at the periphery and both the margins of the carpels were fertile, each bearing one ovule. At present, on the other hand, there is only one ovule to each carpel and this receives its vascular supply in a characteristic manner (see page 77). How this condition could have been derived from that exhibited in Fig 5, *a, b* is shown in Fig 5, *c-f*. The main evidence for such an interpretation comes from a consideration of the nature of the vascular bundles marked as X and Y in Fig 2, *i*. There are, however, two probabilities: either they are placental strands equivalent to those seen in Fig 5, *b* or they are true stelar bundles.

I am inclined to the first view and believe that they are formed by the fusions of the ventral bundles of the two adjacent carpels. Their double nature is unfortunately not quite evident in their origin, although that does not necessarily weaken the argument, for, many cases are on record where the placental strands arise as single bundles instead of double. As a matter of fact the fusion of the ventral bundles has become too deep seated in these cases to allow them to be seen as separate bundles (see Eames, 1931, Dawson, 1936, Puri, 1939). A little higher up, however, they give clear evidence of their double nature, for each of them splits up into two bundles (Fig 2, *j-k*, X₁, X₂ and Y₁, Y₂) which then separate apart. What is still more important in this connection is that the two sister bundles enter into different carpels. For instance, the bundle Y₁ (Fig 2, *l-m*) passes out into the carpel whose ovule is supplied by X₁ coming from the opposite side. In other words this means that X₁, X₂ and similarly Y₁, Y₂ belong to different carpels and hence the parent bundles X and Y are also compound bundles formed in the manner in which they are usually produced in ovaries with parietal placentation (*cf* Fig 5, *a-b*).

This interpretation offers a convincing explanation of how a carpel comes to possess only one ovule. The placental strands X and Y split up into their components X₁, X₂ and Y₁, Y₂ which are marginal bundles of the adjacent ends of the carpels. From one side only one marginal bundle and consequently only one margin⁴ proceeds toward the centre and fuses there with the one coming from the other side. It can be seen from the distribution of the bundles that the two margins fusing in the centre belong to different carpels and that the other margin of each carpel remains in the periphery and becomes sterile in course of time (Fig 5, *c-f*).

⁴ For, the course of the vascular bundles of the margins of the carpels is very well regarded as representing the course of the latter in which they run (see Eames and Wilson, 1928).

Recently Abbe (1938) has traced an interesting series of reduction in the number of ovules in the Betulaceæ where also the gynæceum is bi-carpellary. He has reported 4- to 1-ovuled ovaries although the 2-ovuled condition is the most common. In the 4-ovuled condition two ovules are borne on each carpel and hence both margins of the carpels are fertile. In some cases he found only three ovules and thus he explained by assuming that one margin of one of the two carpels became sterile by losing its ovule. Where there are only two ovules, one to each carpel, they may be borne on either adjacent or diagonally opposite margins of the two carpels. Thus one margin of each carpel has become sterile. In 1-ovuled ovaries one margin of only one carpel remains fertile and bears the single ovule. In *Garcinia livingstonii* the 2-ovuled condition is well established and it is only the diagonally opposite margins of the carpels that have retained their fertility.

According to the second probability X and Y would be regarded as stelar bundles and X_1 and Y_1 as the placental strands derived from them, but this involves certain difficulties which cannot be conveniently explained. If X_1 were a true placental strand, being formed by the fusion of the ventrals belonging to two different carpels, its passing out bodily into the ovule of one carpel would be very unusual. The same can be said of Y_1 . Again, the bundles X_2 and Y_2 supply a different set of carpels from that supplied by their sister bundles. In other words this means that the sister bundles X_1 , X_2 and Y_1 , Y_2 belong to different carpels. This is more clearly understandable on the basis of the first interpretation. Besides, this view does not give any explanation of how a carpel came to possess a single ovule.

Summary

1 The flowers examined are all carpellate ones, having about 15 staminodes which often show development up to the reduction division of the microspore-mother cells.

2 The study of vascular supply does not give any clue into the nature of the disc but it may be a luxuriant outgrowth of the torus.

3 The vascular supply presents evidence for the fact that the placental region of the ovary is formed by the fusion of only two margins of two different carpels, and not four as we should expect in axile placentation, and that the remaining two margins lie in the periphery and have become sterile, so that each carpel bears only one ovule.

4 Development of the embryo-sac proceeds after the *Normal*-type.

It would be unfair to express my gratitude to my teacher, Dr. P. Maheshwari, without adding that thanks to him must not be interpreted as an attempt to claim his support for *all* the statements made in the paper.

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N.B.—Papers marked with an asterisk were not available to me.

TETRASTICHUS SOKOLOWSKII KURDJ (FAMILY . EULOPHIDÆ)—A LARVAL PARASITE OF *PLUTELLA* *MACULIPENNIS* IN SOUTH INDIA

BY M C CHERIAN, B A , B Sc , D I C

AND

MOHAMED BASHEER, B Sc (ALIG)

(Agricultural Research Institute, Coimbatore)

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Introduction

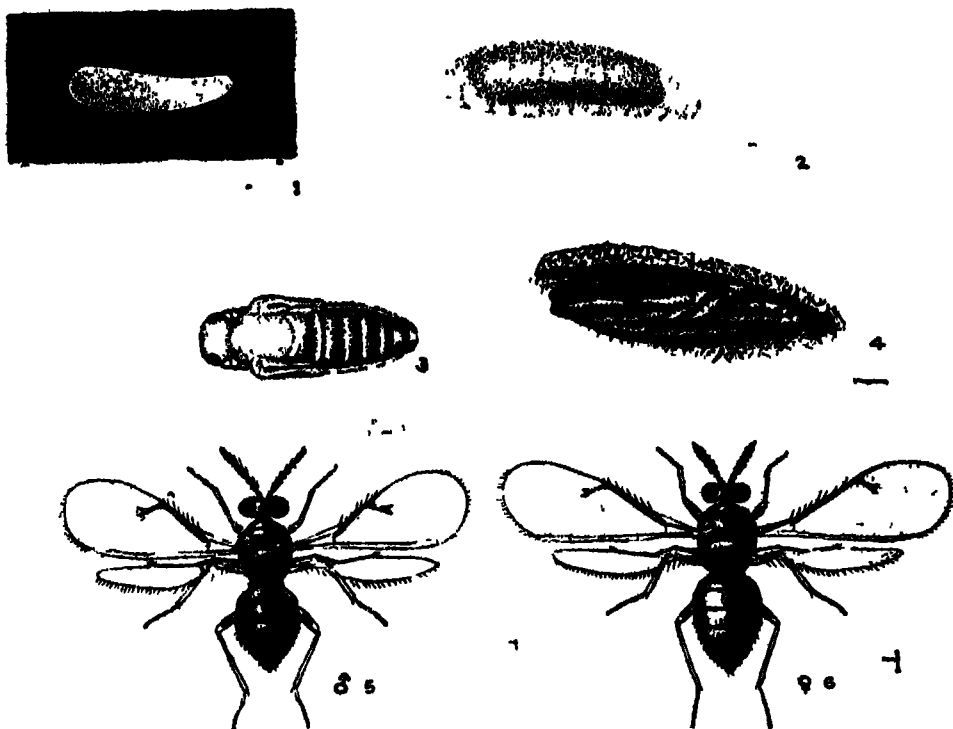
Plutella maculipennis Curtis—a pest of cabbage and other cruciferous plants in different parts of the world—is subject to the attack of a number of natural enemies. In Europe, the most important parasite is an Ichneumonid *Angitia plutellæ* Vier while *Diadromus collares* Grav is also found in some numbers. In America, according to Marsh,¹ in addition to the former, the larvæ are parasitised by *Meteorus* sp , *Mesochorus* sp , and *Microplitis* sp , which, however, are found only in very small numbers. In North India, *Angitia fenestratis* Homlg and *Diadromus collares* Grav have been recorded by Dutt² as parasites of the caterpillars. In South India, the authors have, for the first time, observed two parasites—*Brachymeria excarinata* Gahan and *Tetrastichus sokolowski* Kurdj attacking the pupæ and caterpillars respectively. A paper on the former by the authors³ was published in the *Proceedings of the Indian Academy of Sciences*. The present paper deals with the latter and gives an account of the habits and life history of the parasite and its incidence.

Description of the Parasite

Tetrastichus sokolowski belongs to sub-family Tetrastichinæ, family Eulophidæ. Since the original description of the parasites by Kurdjumov⁴ is not available the short description given below will, it is hoped, be found useful in identifying the wasp.

Female (Fig 6)—Length about 1 to 1.5 mm , antennæ brown ; antennal pedicel about slightly longer than the length of the first funicle joint ; two ring-joints which are visible only when mounted in balsam and under high magnification , the first funicle joint slightly smaller than the two succeeding ones which are sub-equal , the first funicle joint slightly shorter and broader than the pedicel, and, the succeeding two are equal in length but broader than the pedicel ; club three-jointed broader than the funicle

and slightly longer than the two preceding funicle joints, its third joint small, sub-marginal vein with three bristles slightly above the base, marginal vein with nine long bristles, post-marginal vein obsolete, head, thorax and abdomen deep green, abdomen flat and ovate, all coxæ con-colourous with the thorax, remainder of the legs pale yellow, femur green in the middle, apical tarsal joints fuscous



Tetrastichus sokolowskii Kurdj

- | | | | |
|---|-------|---|-------------------------|
| 1 | Egg | 4 | Host pupa showing grubs |
| 2 | Grub | 5 | Male parasite |
| 3 | Pupa. | 6 | Female parasite |

Male (Fig 5) —Length about 1 mm Similar to the female except the antennæ with four funicle joints, the antennal club is more elongate and not broader than the funicle joints, the middle club joint is the largest

Habits of the Parasite

The parasite emerges by biting a small hole at any portion of the host pupa. Copulation takes place soon after the emergence of the parasite. The female wasp first feels the presence of the host larva with her antennæ and then gets upon its dorsal side. When the larva stops moving the female

TABLE I
Statement of composition of ten mated females of *Tetrastichus sokolowskii* Kurdj
(Only one Larva was exposed daily)

No. of females	Pair No 1			Pair No 2			Pair No 3			Pair No 4			Pair No 5			Pair No 6			Pair No 7			Pair No 8			Pair No 9			Pair No 10		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	13			8			10			1			13			10			9			11						1		
2	8			11			7						15			10			7						1			1		
3	8			10			20									1						10						10		
4	7			10												1			20						1			1		
5	16						11									1						10						17		
6				11												1									1			1		
7				12												1						14						15		
8	13			13			11						14			15			15			12						9		
9				10												11			11						1			1		
10							1						18			10			7			9					11			
11				13												7			10			5					7			
12				7												15			14						1			5		
13																10			13			7						11		
14				5												11			11						1					
15				3												9			6			9						1		
16							1											1												
17																														
18																														
Total	66	4	113	3	2	53	2	2	92	1	2	89	5	60	6	127	5	123	7	87	5	1	85	7						

A = Number of Parasites emerged daily B = Number of Moths emerged daily C = Number of Pupae shrunk daily.

takes a convenient position, bends her abdomen, stretches her thin delicate brown ovipositor and thrusts it into the body of the larva and drops her eggs. Eggs are laid even on the day of emergence. The female oviposits generally in the body of the grown-up larvæ but larvæ even six days old are accepted. A parasitised larva is found to pupate the next day after oviposition, irrespective of its age.

Table I gives the egg-laying records of ten mated females. It will be seen from the table that the maximum number of eggs laid by a female was 127 in her life-time and 25 on a single day. When a larva was exposed to a number of parasites more than one parasite was seen ovipositing and the maximum number of parasites that emerged from such single pupa was 39.

Table II shows the number of parasites that emerged from 33 host pupæ collected from the field and the maximum is noted to be only 13.

TABLE II

Statement showing the maximum number of parasites that emerged from host pupæ collected from the field

Serial number	Date of collection of pupæ	Total number of parasites from one host pupa	Sex of the parasites	
			Male	Female
1	16—8—1937	0	1	5
2	20—8—1937	6	1	5
3	2—9—1937	5	1	4
4	2—9—1937	9	1	8
5	2—9—1937	9	1	8
6	2—9—1937	13	2	11
7	2—9—1937	13	2	11
8	2—9—1937	11	2	9
9	2—9—1937	5	1	4
10	2—9—1937	4	1	3
11	3—10—1937	13	3	10
12	3—10—1937	9	1	8
13	3—10—1937	6	0	6
14	3—10—1937	9	1	8

TABLE II—Contd

Serial number	Date of collection of pupae	Total number of parasites from one host pupa	Sex of the parasites	
			Male	Female
15	10-10-1937	7	1	6
16	10-10-1937	12	2	10
17	10-10-1937	12	2	10
18	10-10-1937	12	2	10
19	10-10-1937	11	1	10
20	10-10-1937	9	3	6
21	10-10-1937	9	2	7
22	1-11-1937	12	1	11
23	1-11-1937	13	2	11
24	1-11-1937	13	2	11
25	1-11-1937	5	1	4
26	1-11-1937	9	2	7
27	1-11-1937	10	1	9
28	1-11-1937	12	2	10
29	1-11-1937	9	3	6
30	1-11-1937	9	2	7
31	1-11-1937	5	1	4
32	10-11-1937	9	1	8
33	10-11-1937	11	1	10

Parthenogenesis is quite common in the species, the progeny in such cases being males

Longevity of Adults

Table III gives the longevity records of 88 adults. The parasites were kept in 3" × 1" tubes and fed with honey solution. It will be seen from the table that the maximum longevity in the case of a female which mated and laid eggs in captivity and was fed with honey solution, was 20 days while one which was fed but did not lay eggs lived as many as 34 days. Parasites kept without food did not live for more than 4 days.

TABLE III
Longevity records of Tetrastichus sokolowsku Kurdj

Egg-laying parasites with food						Parasites not laying eggs with food						Parasites without food					
Serial number	Emerged on	Died on	Sex of the adult	Number of days lived	Serial number	Emerged on	Died on	Sex of the adult	Number of days lived	Serial number	Emerged on	Died on	Sex of the adult	Number of days lived	Serial number		
1	1-12-1935	11-12-1935	F	10	1	18-8-1937	25-8-1937	M	7	1	8-9-1937	10-9-1937	M	2	1		
2	1-12-1935	15-12-1935	M	14	2	31-8-1937	12-9-1937	M	12	2	8-9-1937	10-9-1937	M	2	2		
3	1-12-1935	13-12-1935	F	12	3	31-8-1937	12-9-1937	F	12	3	8-9-1937	10-9-1937	F	2	3		
4	1-12-1935	13-12-1935	M	12	4	31-8-1937	12-9-1937	F	12	4	8-9-1937	10-9-1937	F	2	4		
5	1-12-1935	18-12-1935	F	17	5	31-8-1937	12-9-1937	F	12	5	8-9-1937	10-9-1937	F	2	5		
6	1-12-1935	21-12-1935	M	20	6	31-8-1937	4-9-1937	M	4	6	8-9-1937	10-9-1937	F	2	6		
7	8-12-1935	21-12-1935	M	13	7	31-8-1937	15-9-1937	F	15	7	8-9-1937	11-9-1937	M	3	7		
8	8-12-1935	17-12-1935	F	9	8	31-8-1937	15-9-1937	F	15	8	8-9-1937	11-9-1937	F	3	8		
9	7-10-1937	22-10-1937	M	15	9	31-8-1937	15-9-1937	F	15	9	8-9-1937	11-9-1937	F	3	9		
10	7-10-1937	17-10-1937	F	10	10	31-8-1937	15-9-1937	F	15	10	8-9-1937	11-9-1937	F	3	10		
11	7-10-1937	21-10-1937	M	14	11	31-8-1937	16-9-1937	F	16	11	8-9-1937	11-9-1937	F	3	11		
12	7-10-1937	23-10-1937	F	16	12	31-8-1937	16-9-1937	F	16	12	9-9-1937	10-9-1937	M	1	12		
13	7-10-1937	21-10-1937	M	14	13	8-9-1937	15-9-1937	M	7	13	9-9-1937	12-9-1937	F	3	13		
14	7-10-1937	25-10-1937	F	18	14	8-9-1937	16-9-1937	F	8	14	9-9-1937	12-9-1937	F	3	14		
15	7-10-1937	22-10-1937	M	15	15	13-9-1937	16-10-1937	F	33	15	9-9-1937	12-9-1937	F	3	15		
16	7-10-1937	22-10-1937	F	15	16	13-9-1937	16-10-1937	F	33	16	9-9-1937	12-9-1937	F	3	16		

Average longevity for		14.4 13.6						18.4 17						2 3	
		M F													
17	7-10-1937	22-10-1937	M	15	17	13-9-1937	16-10-1937	F	33	17	9-9-1937	12-9-1937	F	3	
18	7-10-1937	20-10-1937	F	13	18	13-9-1937	16-10-1937	F	33	18	9-9-1937	12-9-1937	F	3	
19	7-10-1937	19-10-1937	M	12	19	13-9-1937	13-10-1937	F	30	19	9-9-1937	12-9-1937	F	3	
20	7-10-1937	23-10-1937	F	16	20	13-9-1937	13-10-1937	F	30	20	9-9-1937	12-9-1937	F	3	
					21	13-9-1937	13-10-1937	F	30	21	9-9-1937	13-9-1937	F	4	
					22	13-9-1937	17-10-1937	M	34	22	9-9-1937	13-9-1937	F	4	
					23	22-9-1937	13-10-1937	M	21	23	21-9-1937	22-9-1937	M	1	
					24	22-9-1937	13-10-1937	M	21	24	21-9-1937	22-9-1937	F	1	
					25	22-9-1937	13-10-1937	M	21	25	21-9-1937	22-9-1937	F	1	
					26	22-9-1937	13-10-1937	M	21	26	21-9-1937	24-9-1937	M	3	
					27	25-9-1937	29-10-1937	F	34	27	21-9-1937	24-9-1937	F	3	
					28	25-9-1937	29-10-1937	F	34	28	21-9-1937	24-9-1937	F	3	
					29	25-9-1937	29-10-1937	F	34	29	7-10-1937	10-10-1937	M	3	
					30	10-10-1937	21-10-1937	M	11	30	7-10-1937	10-10-1937	M	3	
					31	10-10-1937	21-10-1937	M	11	31	7-10-1937	10-10-1937	F	3	
					32	10-10-1937	21-10-1937	F	11	32	8-10-1937	9-10-1937	M	1	
					33	10-10-1937	21-10-1937	F	11	33	8-10-1937	9-10-1937	F	1	
					34	10-10-1937	21-10-1937	F	11	34	8-10-1937	9-10-1937	F	1	

Incidence of Parasites

Table IV shows the percentage of parasitisation of pupæ collected from the field. This varied from 18.2 to 68.5, the highest being in November.

TABLE IV

Percentage of parasitised pupæ collected from the field

	August 1937	September 1937	October 1937	November 1937
Number of pupæ collected	192	168	239	54
Number of parasitised pupæ	35	83	110	37
Percentage of parasitisation	18.2	49.4	46.0	68.5

Life-History of the Parasites

The egg (Fig 1) is generally translucent, hardly visible to the naked eye in the fluid contents of the larva. The egg measures 0.2 to 0.25 mm by 0.05 to 0.07 mm. It is cylindrical, very slightly curved, one end being more pointed than the other. The eggs are laid in groups in the body of the caterpillar. The egg period has been noted to be less than 2 days.

The just hatched grub is transparent. As it grows it changes its colour to pale green and then to pale yellow. The fully grown grub (Fig 2) measures, from 1-1.5 mm. by 0.5 mm. It is thickest in the middle with both ends narrowing down. The egg and larval period together vary from 5 to 8 days. Fig 4 shows a host pupa showing grubs.

The pupa (Fig 3) is boat-shaped, anterior end being broader. It measures 1-1.5 mm by 0.5 mm. The newly formed pupa is pale yellow in colour. On the second day the eyes of the pupa turn slightly pink and abdomen slightly dark. On the third and fourth day these colours deepen and the thorax and abdomen become dirty brown. On the fifth day the whole pupa darkens. The pupal period varies from 5 to 7 days.

Total Life-Cycle

Table V gives the life-cycle of 55 adults. It is seen from the table that the parasite generally takes 10 to 15 days to complete its life-cycle from egg to adult. The temperature and humidity at which the parasites were studied are given in Table VI.

TABLE V
Detailed life-history records of *Tetrastichus sokolowski* Kurdj

Serial number	Date of <i>Plutella</i> larva exposed to the parasites	Date of pupation of the grub	Egg and larval periods together in days	Date of emergence of the adult	Pupal period in days	Total life cycle from egg to adult in days	Sex of the parasites
1	3—9—1937	8—9—1937	5	13—9—1937	5	10	F
2	3—9—1937	8—9—1937	5	13—9—1937	5	10	F
3	3—9—1937	8—9—1937	5	13—9—1937	5	10	F
4	3—9—1937	8—9—1937	5	13—9—1937	5	10	F
5	4—11—1937	12—11—1937	8	17—9—1937	5	13	M
6	4—11—1937	12—11—1937	8	17—9—1937	5	13	M
7	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
8	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
9	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
10	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
11	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
12	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
13	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
14	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
15	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
16	4—11—1937	12—11—1937	8	17—9—1937	5	13	M
17	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
18	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
19	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
20	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
21	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
22	4—11—1937	12—11—1937	8	17—9—1937	5	13	F
23	5—11—1937	12—11—1937	7	17—9—1937	5	13	M
24	5—11—1937	12—11—1937	7	17—9—1937	5	12	M
25	5—11—1937	12—11—1937	7	17—9—1937	5	12	M
26	5—11—1937	12—11—1937	7	17—9—1937	5	12	F
27	5—11—1937	12—11—1937	7	17—9—1937	5	12	F

TABLE V—*Contd*

Serial number	Date of <i>Plutella</i> larva exposed to the parasites	Date of pupation of the grub	Egg and larval periods together in days	Date of emergence of the adult	Pupal period in days	Total life cycle from egg to adult in days	Sex of the parasites
28	5-11-1937	12-11-1937	7	17 9 - 1937	5	2	F
29	5- 11-1937	12 11 1937	7	17- 9- 1937	5	12	F
30	5 -11- 1937	12-11-1937	7	17 - 9 -1937	5	12	F
31	5- 11 1937	12- 11 1937	7	17- 9-1937	5	12	F
32	5-11 1937	12--11--1937	7	17- 9 -1937	5	12	F
33	5- 11 1937	12 11 -1937	7	17- 9-1937	5	12	F
34	5-11- 1937	12 11 -1937	7	17 9 - 1937	5	12	F
35	5-11- 1937	12-11- 1937	7	17 9 -1937	5	12	F
36	5- 11 1937	12 11 1937	7	17- 9-1937	5	12	F
37	5- 11 1937	12 11-1937	7	17 9 1937	5	12	F
38	5-11-1937	12-11-1937	7	17 - 9-1937	5	22	F
39	5-11 1937	12 - 11 1937	7	17- 9 -1937	5	12	F
40	5 11-1937	13- 11-1937	8	18 9 -1937	5	13	M
41	5 11-1937	13 11 1937	8	18- 9-1937	5	13	F
42	5- 11 1937	13-11-1937	8	18- 9-1937	5	13	F
43	5- 11 1937	13 11- 1937	8	18- 9- 1937	5	13	F
44	5 -11- 1937	13- 11-1937	8	18 - 9-1937	5	13	F
45	5-11- 1937	13- 11-1937	8	18 9 -1937	5	13	F
46	5- 11- 1937	13- 11 1937	8	18 - 9-1937	5	13	F
47	6-11 1937	14 11 -1937	8	21 -11- 1937	7	15	M
48	6- 11 -1937	14 -11- 1937	8	21 -11-1937	7	15	F
49	6-11- 1937	14-11 1937	8	21-11-1937	7	15	F
50	6-11- 1937	14 -11- 1937	8	21-11-1937	7	15	F
51	6-11-1937	14- 11-1937	8	21-11-1937	7	15	F
52	6- 11- 1937	14-11-1937	8	21- 11-1937	7	15	F
53	6 11 -1937	14-11-1937	8	21-11-1937	7	15	F
54	6-11-1937	14-11-1937	8	21-11-1937	7	15	F
55	6-11- 1937	14-11-1937	8	21 -11-1937	7	15	F

TABLE VI

Temperature and humidity at which the life-history of the parasite was studied

Temperature and humidity	December 1937	August 1937	September 1937	October 1937	November 1937
Mean minimum in shade	65° 8' F	71° 2' F	70° 8' F	70° 6' F	69° 0' F
Mean maximum in shade	84° 9' F	88° 7' F	89° 5' F	86° 0' F	82° 2' F
Mean humidity at 8 hours	75.1%	74.5%	72.0%	77.4%	81.4%

Other Species of Tetrastichus in South India

In addition to *Tetrastichus sokolowskii* the following species are found in South India —

Tetrastichus ophiussæ Craw on *Achæa janata* larva, *Tetrastichus ayyari* Roh on *Chilo simplex* pupa on sorghum, *Tetrastichus schænobii* R on *Schænobius incertellus* egg mass, *Tetrastichus colomanni* Craw on *Aspidomorpha mihares*, larva, *Tetrastichus nyemilavus* Roh on cholam maggot, *Tetrastichus coimbatorensis* Roh on cholam gall-fly, *Tetrastichus isaaci* Roh on galls on *Macrena arneria* probably on gall-flies, *Tetrastichus okawus* Roh from cumbu grains probably on gall-flies and *Tetrastichus echthrus* Crawford hyper-parasite on *Apanteles* on larva on *Daincha*

Summary

Tetrastichus sokolowskii is a parasite of *Plutella maculipennis*. The wasp lays eggs inside the caterpillar which pupates the next day. The maximum number of eggs laid by a female was 127, the maximum number of parasites emerging from a single pupa was 25. Cases of parthenogenesis have been met with. The life-cycle of the parasite varied from 10–15 days. The maximum longevity was 34 days in the case of a female. The percentage of incidence of the wasp ranged from 18.2 to 68.5, the highest being in November.

Acknowledgments

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CUTICULAR STUDIES OF MAGNOLIALES

By H S RAO, M Sc

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Introduction

At a discussion on the origin of angiosperms held at Amsterdam in September 1935 it was casually suggested that the cuticles of Magnoliales may be worthy of investigation as they may resemble those of the Bennettitales. It therefore occurred to Professor Sahn that the question might be taken up by some one in the East as the material would be more easily accessible there. So it was at the suggestion of Professor Sahn that I took up the present work at Lucknow.

The question of the origin of the angiosperms is still by no means finally settled. A large number of investigators have tackled the problem from diverse angles. The Bennettitalean ancestry seems rather to have been set aside, more and more workers seem to be fascinated by the discovery of the Caytoniales by H H Thomas (1925 and 1931). While some of the Bennettitalean remains are almost complete as regards the various organs of the plants, the Caytoniales have so far definitely provided only the reproductive organs. *Sagenopteris* has been suspected to belong to Caytoniales, and there are strong reasons for this correlation though as yet it has not been demonstrated to be an absolute fact. The cuticle of *Sagenopteris* is said to resemble that of a mesophytic angiosperm and the stomata to be of the angiospermous

type (H H Thomas, 1931, p 651) The stems of Caytoniales have still to be discovered Thus we have still to await further discoveries of the other organs of the plant body Further, Harris (1933) has given reasons to say that the Caytoniales cannot be considered to be angiosperms in the literal sense as Hamshaw Thomas was inclined to call them in his first paper (H H Thomas, 1925) Harris has given reasons for the belief that the stigma of Caytoniales gave direct access to pollen grains (see also Johri, 1935, Sahní, 1936)

In the meantime comparative studies of the Bennettitales and Magnoliales need not be closed Apart from the resemblances in the floral structure which seemed striking to the early workers, the wood anatomy of the two groups has been lately discussed (Gupta, 1934) It may not be just a chance that the only homoxylous angiosperms are found among the Magnoliales, and resembling the Bennettitalean structure Professor Sahní (1932) has already revived the view of the Bennettitalean ancestry of angiosperms in these words "The theory according to which the angiosperms have been derived from some gymnospermous group like the Bennettitales through the Magnoliaceae or some related extinct family would thereby gain distinct support "

The external resemblances of the leaves of Bennettitales with those of cycads led early workers to call the former fossil cycads But Florin (1931) has found a means of distinguishing the Bennettitales from the true fossil cycads by their epidermal studies *

Florin has found that the Bennettitales possess a more highly evolved type of cuticular structure, i.e., the "syndetocheile" (Florin, 1933) or compound-lipped type That only the Bennettitales and some members of the Gnetales among all the living and fossil gymnosperms show the advanced type of cuticular structure found also in the angiosperms may not be a mere chance As we do not know much about the fossil history of the Gnetales, attention may again be focussed on the Bennettitales

The present work was undertaken to compare the cuticular features of the Magnoliales with those of the Bennettitales on the one hand and the "haplocheile" gymnosperms on the other While from this point of view almost the whole lot of angiosperms have been untouched a beginning has been made in this study of one of the primitive families of angiosperms, the Magnoliales

Material and Methods

The material available to me represents almost all the genera of the cohort Magnoliales of Hutchinson (1926, p 81)

* Thomas and Bancroft (1913) had also made this distinction though they did not have so comprehensive an idea of the Bennettitalean cuticular structure as Florin.

Most of the material was obtained from the Sibpur Herbarium, Royal Botanic Garden, Calcutta. My material was taken mostly from old collections, some over fifty years old. It consisted of bits of leaves from the authenticated duplicate sheets of the herbarium. I have relied on the final identifications that were noted on the herbarium sheets. I am thankful to Messrs Biswas and Calder for kindly allowing me to take the bits of leaves from the authenticated duplicates.

Leaves of a few genera unrepresented in the Sibpur Herbarium were obtained by Prof. Sahni from Mr. Humphrey Gilbert-Carter, Director, University Botanical Garden, Cambridge, to whom also my thanks are due.

The following types have been examined —

Magnolia, 13 spp., *Liriodendron*, 1 sp., *Manghetia*, 4 spp., *Michelia*, 10 spp., *Talauma*, 9 spp., *Aromadendron*, 1 sp., *Drimys*, 2 spp., *Illicium*, 5 spp., *Schizandra*, 3 spp., *Kadsura*, 4 spp., *Trochodendron*, 1 sp., *Tetracentron*, 1 sp., *Euptelea*, 1 sp., and *Cercidiphyllum*, 1 sp.

Lactoris, *Himantandra* and *Zygogynum* were unrepresented.

Cuticular preparations were made in the same way as with the fossils, so as to have a uniform method. The bits of leaves were first soaked in warm water so that the dried up cells might regain the original shape, and then immersed in Schultze's macerating fluid. They were then treated with a solution of potassium hydroxide. After this treatment only the cuticles were left unaffected. On washing in water and shaking vigorously in a tube the cuticles were separated from the oxidised tissues. They were then stained in Bismarck Brown and permanent slides were prepared.

Microtome sections were prepared in some cases, but it was difficult to obtain good sections as the cuticles were brittle and the leaves hard. But enough sections were got to correlate the surface and sectional views of stomata. Hand sections of fresh material of *Michelia champaca* were quite satisfactory. With the oil-immersion lens it was possible to study the hand-sections by focussing different levels with the fine adjustments of the microscope. Modelling with plastic wax was of use in elucidating the structures.

Cuticles of more than one leaf could not be examined in the majority of cases as the material consisted of small bits of single mature leaves.

Previous References

By far the most important work on the cuticles of living gymnosperms and the Bennettitales is by Rudolf Florin. In his memoir, "Untersuchungen zur Stammesgeschichte der Coniferales und Cordaitales" (1931), he has given a summary of all the essential features described in the previous works.

Among the earlier works Porsch's (1905) seems to be the most important as it is a comprehensive study of the epidermal structure with a view to establish phylogenetic relationships among plants. But he has argued too much for the influence of ecological factors on the epidermal structure of plants (see Thomas and Bancroft, 1913, p. 1, and Florin, 1931, pp. 180-94). Conclusions of recent workers seem to show that the cuticular studies cannot be utilised on a wide scale for the solution of phylogenetic problems, but can be well applied for generic and group demarcations within restricted limits (Florin, 1931 Introduction).

The cuticular structure of the angiosperms is practically uninvestigated from the phylogenetic point of view. Florin (1931, p. 523) remarks that a detailed and extensive investigation of the epidermal structure of the angiosperms has to be taken up before conclusions as to the phylogenetic and generic affinities can be drawn.

On the basis of the ontogenetical studies of the stomata in the Coniferales and Gnetales Florin (1931 and 1934) has distinguished two essentially different types of development. The primitive type met with in the Coniferales, Ginkgoales, Cycadales and *Ephedra*† is called by him "haplocheile" or simple-lipped (Florin, 1933) and that found in *Welwitschia* and *Gnetum* the "syndetocheile" or compound-lipped. Among the fossil forms he has found the latter type only in the Bennettitales.

In the haplocheile type the mother cell of the guard cells divides but once forming the two guard cells. The subsidiary cells are formed by the surrounding epidermal cells which were contemporaneous with the mother cells of the guard cells.

In the syndetocheile type (which is regarded as more advanced because there is greater differentiation in the epidermis) the mother cell first gives rise to two cells (see Figs. 59, 60). One of these cuts off a third middle cell. The latter is the mother cell of the guard cells. Thus both the guard cells and the lateral subsidiary cells are the result of divisions of a single original mother cell.

From an examination of the illustrations given at the end of the paper it will be clear that the Magnoliales (except in the case of the two plants to be mentioned below) possess the syndetocheile type of stomata.

Florin in his study of the Coniferales has taken into consideration the epidermal structure in the seedlings and in the cotyledons. From this study he comes to the conclusion that more variations are seen in the cotyledons

† *Casuarina* among the angiosperms is said to possess the gymnosperm type—Zimmermann (1926, p. 100) after Porsch.

than in the mature leaves, though within narrow limits. The epidermis of both the cotyledons and the foliage leaves is little influenced by the external factors (Florin, 1931, p. 221).

Description of the Cuticles

General—From the following account the epidermis of the Magnohales is seen to possess a uniform character. The stomata belong (except in *Cercidiphyllum japonicum* and *Euptelea franchetii*) to the Bennettitalean or the syndetocheile type of Florin. The two lateral subsidiary cells are about the same size and evidently these and the guard cells are the result of divisions of a single mother cell. The polar subsidiary cells are only adjacent cells of the epidermis and have no immediate genetical relation with the guard cells.

The lateral subsidiary cells in some cases are divided into two (4 in *Schizandra elongata*) by a transverse division, e.g., spp. of *Schizandra*, *Kadsura*, etc. A longitudinal division occurs in many cases, resulting in a lateral subsidiary cell and a "Kranzzelle" of Florin outside it, e.g., *Galauma andamanica*, *Magnolia Henryi*, and etc. As in the case of the Bennettitales the stomata are not much sunk below the general level of the epidermis. The epidermal cells in the stomatiferous areas are polygonal or irregular in shape, with the walls sinuous to gently wavy and in a few cases nearly straight (see table). Those on the veins and at the edge of the leaf are elongated and somewhat rectangular (Figs. 28 & 29).

Stomata as a rule occur only on the under-surface of the leaves. A narrow zone at the edge of the leaf is without stomata, as also over the veins. Hair-scars when present are observed usually on the lower surface and in *Kadsura scandens* (Fig. 105) on the upper surface also. The cuticle over the veins and at the edge of the leaf is somewhat thicker than in the stomatiferous areas.

Simple pits are sometimes seen in the epidermal cells (Figs. 67, 85, 93, etc., also see table). They are few in number and have no definite arrangement as in *Cycas* (Thomas and Bancroft, 1913, p. 161).

The stomata are orientated in no definite manner as regards the longitudinal axis.

In *Cercidiphyllum* the cuticular edge around the stomatal opening forms a vestibule above the stomatal pore (Fig. 116). This vestibule ("Vorhof") is characteristic of the Bennettitales (*Cercidiphyllum* is otherwise of the gymnospermous haplocheile type). Among the gymnosperms such a cuticular border is said to occur in *Cordaites* and *Sciadopitys* (Florin, 1931, p. 510).

The number of stomata per unit area seems fairly constant at different parts of the individual leaf. (In most cases, however, only small bits of leaves were available for examination.) In the accompanying table averages

Epidermal features of the Magnoliales

Serial number	Name of plant	No of stomata per unit area (= 10 sq mm)		No of lateral subsidiary cells	Epidermal walls						Pits in outer walls of epidermal cells	Other features	
		Average for species	Average for genus		Lower epidermis		Upper epidermis						
					Sinuous	Slightly wavy	Straight	Sinuous	Slightly wavy	Straight			
1	<i>Magnolia nitida</i>	76		2		x					x		
2	<i>M. mangrove</i>	72		2		x					x		
3	<i>M. Henry</i>	33		2, long divn in one subs cell	x				x				
4	<i>M. eboaria</i>	30		2	x				x				
5	<i>M. rostrata</i>	46		2, long divn in one subs cell		x				x			
6	<i>M. Champsoni</i>	54		2	x				x				
7	<i>M. grandiflora</i>	56		2		x			x				
8	<i>M. conspicua</i>	52	65	2, long divn in one subs cell		x	x		x	x			
9	<i>M. Liliacarpa</i>	87		Do		x			x				
10	<i>M. tabernaem</i>			2		x			x		x		
11	<i>M. pterocarpus</i>	63		2		x			x		x		
12	<i>M. hypoleuca</i>	98		2		x			x	x	x		
13	<i>M. lobata</i>	109		2		x			x		x		
14	<i>Larodendron telepsifera</i>	65	65	2									
15	<i>Mangifera Hookeri</i>	302		2		x		x				x	

[illegible]

Epidermal features of the Magnoliales—(Contd.)

Serial number	Name of plant	No of stomata per unit area (= 10 sq mm)		No of lateral subsidiary cells	Epidermal walls						Pits in outer walls of epidermal cells	Other features	
		Average for species	Average for genus		Lower epidermis			Upper epidermis					
					Rinuous	Slightly wavy	Straight	Rinuous	Slightly wavy	Straight			
38	<i>Armadillan elegans</i>	85	85	2	x		x		x			x	
39	<i>Dryas winters</i>	30	37	2, Long divn in both-subcells									
40	<i>D. aromatica</i>	43		Do									
41	<i>Illicium Griffithii</i>	35		2		x				x			
42	<i>I. combodianum</i>	17		2	x								
43	<i>I. exaltum</i>	24	25	2, 3, 4 or 5 surrounding cells (3 normal)		x							3 subs cells due to oblique divn in stomas mother cell
44	<i>I. Sinense</i>	24		2	x								Cuticular striations
45	<i>I. neesii</i>	26		2		x				x			
46	<i>Schinus molle</i>	17		2				x	x			x	Cuticular striations
47	<i>S. molle</i>	26	23	2; long divn in one sub-cell, tr divn in the other								x	Do
48	<i>S. elongata</i>	24		2; long. divn in one sub-cell, 1 or 2 tr divns in the other					x				
49	<i>Kadsura conifera</i>	13		2, tr divn in one sub-cell	x								

			2, tr divrs in each sube cell Do						Hairs on both sur- faces Cuticular stratuons
50	<i>K. Randersiana</i>	20	18						
51	<i>K. canadensis</i>	11							
52	<i>K. laevicollis</i>	17		2					
53	<i>Tetradactylon arborescens</i>	28	28	2					
54	<i>Tetradactylon sinense</i>	26	26	2, tr divrs in each sube. cell					
55	<i>Eupatica Franchetii</i>	35	35	4, 6 or 7 cells surrounding the stoma					
56	<i>Cercidiphyllum japonicum</i>			7 cells surround- ing the stoma					

of six readings are given. As can be expected the larger numbers indicate smaller size of the epidermal cells as well as of stomata. It will be seen that the genera of the family Magnoliaceæ of Hutchinson are according to the *generic averages* characterised by a larger number of stomata per unit area than the genera of the other families under his Magnoliales.

This higher frequency in itself has not much phylogenetic significance, except perhaps indicating a general xerophytic habit (see Salisbury, 1928). Prof. Salisbury has introduced the concept of the "stomatal index," i.e., he takes into consideration more the proportion of the epidermal cells which are destined to develop into stomata, than mere stomatal frequencies.

From the accompanying table a striking fact appears which might be worth mentioning: the only forms in which transverse divisions of subsidiary cells are seen are those with fewer stomata per unit area (outside the Magnoliaceæ of Hutchinson). I suggest the possibility that here, where the cells are large, two subsidiary cells give a better mechanical support to the guard cells than only one for their opening and closure movements.

Surface view of cuticles—The constant presence (except in *Cercidiphyllum* and *Euptelea*) of two lateral subsidiary cells is to be marked. Occasionally a longitudinal division takes place in them, generally on one side only, e.g., *Talauma andamanica*, *Michelia montana*, etc. Among the Bennettitales *Nilssoniopsis glandulosa* shows a similar condition (Florin, 1932, p. 3). In the two species of *Drimys* the longitudinal division takes place in both the subsidiary cells resulting in two "Kranzzellen" outside the two subsidiary cells. In some members, e.g., *Kadsura caulisflora*, *K. scandens*, *Tetracentron*, etc., a transverse division takes place in the lateral subsidiary cells, either in one or in both. In such cases the stomata are surrounded by 5 or 6 cells, of which two are polar subsidiary cells. Such large numbers are not to be mistaken for the surrounding cells of haplocheile type. It is only a case of meristematic division going a step further. Occasional transverse division in the lateral subsidiary cells takes place also in some Bennettitales, e.g., *Williamsonia pecten* in one of the subsidiary cells and *Williamsonia Wettsteinii* in both the subsidiary cells—(see Florin 1933, pp. 7 and 12).

In *Illicium evernum* there is the preponderant number of surrounding cells, in which case one of the lateral subsidiary cells curves round one polar end of the stoma and thus comes to function as the polar subsidiary cell also. This is due to an oblique second division of the original mother cell (which results in the mother cell of the guard cells). The normal arrangement of syndetocheile members also occurs here and there. Sometimes a transverse division also occurs in the lateral subsidiary cells. Hence,

although the number of surrounding cells varies from two to four or five, it still conforms to the syndetocheile type and is not to be mistaken for the haplocheile

Pits in the outer walls of the epidermal cells are seen in a few cases, e.g., *Magnolia pterocarpa*, *Talauma villosa*, etc (Figs 67, 85, etc.), but they have no definite peripheral arrangement as in the case of *Cycas*† and are fewer in number

Cuticular undulations on the surface are seen in some cases (*Illicium simonsii*, *Schizandra propinqua*, *Kadsura lanceolata*, etc., see table), and have been shown as dotted lines (Figs 90, 106, see also table). Similar cuticular striations are seen also in *Stangeria* (Thomas and Bancroft, 1913, pp 159-60), but in no other member of the Cycadales

Cercidiphyllum japonicum and *Euptelea franchetii* are the only members among all the genera and species examined which show an important deviation in the cuticular structure. They possess from four to seven subsidiary cells (seven in *C. japonicum* and 4, 6 or 7 in *E. franchetii*) around the stomata. The cells from their shape evidently are only adjoining cells of the epidermis and are not developmentally to be traced to a common origin with the guard cells. This would mean that only the two guard cells result from the division of the original mother cell, thus conforming to the "haplocheile" type of development.

Sectional views of epidermis—Sectional views of the following have been studied—*Michelia champaca*, *Drimys aromatica*, *Liriodendron tulipifera* and *Cercidiphyllum japonicum*

The guard cells are slightly sunken below the general level of the epidermis. In the median transverse section the guard cells are seen to possess two thickenings, one in the dorsal wall and one in the ventral, i.e., adjacent to the mesophyll tissues. The dorsal thickening is absent at the polar ends of the guard cells. It is on account of this that in the surface view there are two clear areas shown at the extremities of the guard cells.

In *Cercidiphyllum* there is a pronounced cuticular projection over the stomatal pore forming an outer vestibule ("Vorhof"). Thomas and Bancroft (1913, p. 182) in their reconstruction of the stoma of *Phyllophyllum pecten* (Bennettitales) have mistaken a similar cuticular border for the dorsal thickening of the guard cells (see Florn, 1931, p. 508). *Sciadopitys* alone among the Coniferales, and *Cordaites* are shown to possess such an outer

† Pittings are said to be absent in the other Cycads (*Microcycas* not examined)—Thomas and Bancroft, 1913, p. 161.

chamber (Florin, 1931, p 112) Toward the polar ends of the guard cells this cuticular edge (in *Cercidiphyllum*) is not so high.

Thus the haplocheile *Cercidiphyllum* combines in itself also the Bennettitalean character, viz, the presence of the vestibular border of the cuticle and the sinuous walls of the epidermal cells § This combination of character is remarkable Engler and Prantl (see Willis, 1919) place *Cercidiphyllaceæ* previous to *Magnoliaceæ* among the *Ranales* and under *Trochodendrineæ* *Trochodendron* is well known to possess homoxylous wood (Gupta, 1934, and literature cited therein) But *Cercidiphyllum*, Prof Sahn informs me, does not possess homoxylous wood *Euptelea*, *Illicium*, *Kadsura*, *Schizandra*, *Michelia*, *Talauma* and *Liriodendron* are also known to possess typical 'vessel-members' (Bailey and Thompson, 1918, p 504)

The various features of the epidermis of the *Magnoliales* are tabulated in the accompanying table for the sake of convenience

Conclusion

The *Magnoliaceæ* have for a long time claimed to be considered as among the most primitive angiosperms Very early (Hallier, 1905, Arber and Parkin, 1907) an attempt was made to seek for their descent through the *Bennettitales* But this was somewhat set aside until revived by Prof Sahn (1932) and later followed up by Gupta (1934) The present investigation shows that at least a few of the members of the *Magnoliales* represent synthetic types *Cercidiphyllum* combines the characters of the angiosperms, the *Bennettitales* and the haplocheile gymnosperms *Euptelea franchetii* possesses the haplocheile type of stomatal development The cycadean character, viz, the cutin striations seen in *Stangeria*, are also found in some members of the *Magnoliales* (*Illicium simonsii*, *Kadsura lanceolata*, etc (see table and Figs 90, 106)

The present study has opened the question of the position of the *Magnoliales* from the point of view of cuticular analysis While the great host of angiosperms remain uninvestigated from this standpoint, this short study indicates that the *Magnoliales* have not been directly derived from the *Bennettitales*, and that synthetic types combining the characters of the *Cycadales*, the other haplocheile gymnosperms and the angiosperms are to be found in this small group This is well in keeping with our anticipations

The general problem of the origin of the angiosperms remains the same. The additional light thrown on it by the cuticular analysis restates the view

§ The *Bennettitales* possess sinuous walls generally exceptions being *Williamsonia spectabilis* Nath., etc. which have straight-walled epidermal cells (Florin, 1933, pp 5, 6)

that the progenitors of the angiosperms have to be sought for in that plexus of gymnosperms which gave rise on the one hand to the Bennettiales, the syndetocheile Gnetales on the other, the haplocheile Magnoliales along a third line, and the majority of the Magnoliales, viz, the syndetocheile ones, along a fourth line of descent, *this last being probably nearest related to the Bennettiales* (from the present point of view) All the homoxylous Magnoliales (Gupta, 1934) possess the syndetocheile type of cuticular structure (*Zygogynum* has not been available to me) This constant combination of homoxily and syndetocheily may have some phylogenetic significance Syndetocheily by itself may not count for much as it is common in the vast majority of the angiosperms

It may be of interest to draw attention to the geographical distribution and systematic position of the two haplocheile genera *Euptelea* and *Cercidiphyllum* The former comprises three species extending from Japan to Bengal and the latter has two species confined to Japan In Engler and Prantl's classification (see Willis, 1919) Trochodendraceæ (to which belongs *Euptelea*) and Cercidiphyllaceæ are the third and fourth families respectively under the Cohort Ranales, while Magnoliaceæ occupies the ninth position The sub-group Trochodendrineæ (i.e., Trochodendraceæ and Cercidiphyllaceæ) is characterised by naked flowers, while Magnolineæ (comprising Magnoliaceæ, etc) possess flowers with perianth From the point of view of the wood-anatomy of homoxylous Magnoliales also the Trochodendron group is considered to be older than the Drimys group (Gupta, 1934, p 93) Thus the aberrant epidermal characters of *Euptelea* and *Cercidiphyllum* seem to go hand in hand with other primitive features already known to exist in the group Trochodendrineæ

It appears from the point of view of the present inquiry that the Magnoliales of Hutchinson do not represent a co-sanguinary group, but indicate a convergence

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I am indebted to Professor Sahnî who suggested to me this work and gave me the necessary literature His suggestions and other help have been invaluable to me in the preparation of this paper My gratitude is due to the authorities of the Lucknow University for awarding me a Research Fellowship during which term a part of this work was done I have great pleasure in particularly acknowledging the laboratory facilities given me by Prof. M. A. Sampathkumaran of Bangalore, and Mr C V Krishna Iyengar of Mysore without whose kindness this work would have remained unfinished

Summary

In the present work the question of the position of the Magnoliales from the point of view of their epidermal features has been taken up. This is rather a new line of inquiry. Florin's criteria, viz., the haplocheile and syndetocheile types of stomatal development have been made use of. According to this test the Magnoliales are seen to comprise a few synthetic forms, some possessing certain Cycadean features, the great majority the Bennettitalean, and a few the haplocheile gymnosperm features.

The aberrant epidermal features of *Euptelea* and *Cercidiphyllum* seem to be correlated with other primitive features of Trochodendraceae already known.

The view has again been expressed that the progenitors of the angiosperms have to be sought for in that plexus of gymnosperms which gave rise to the Bennettiales, the syndetocheile Gnetales, the haplocheile Magnoliales and the majority of the Magnoliales (i.e., syndetocheile) along different lines of descent, the last being probably nearest related to the Bennettiales.

From the point of view of epidermal studies, the Magnoliales do not represent a co-sanguinary group, but indicate a convergence.

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EXPLANATION OF PLATES

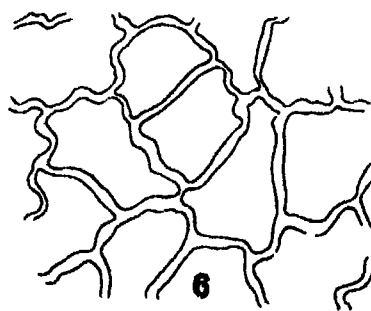
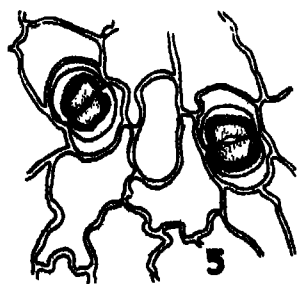
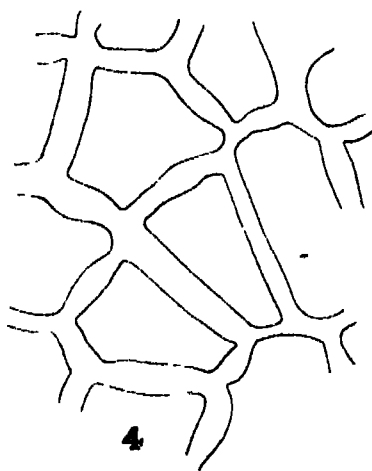
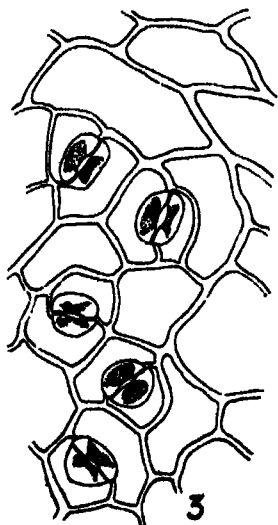
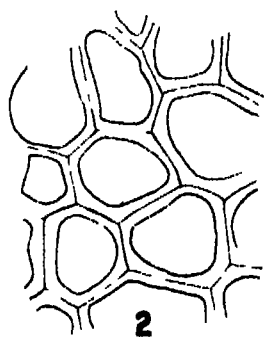
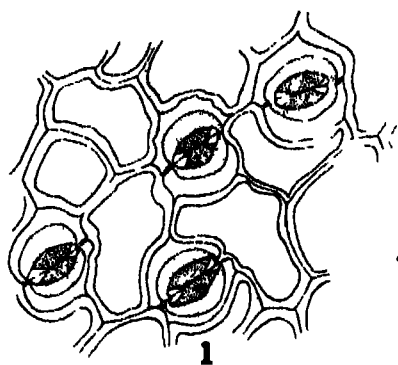
(Names of countries marked against the species refer to the source of the herbarium sheets)

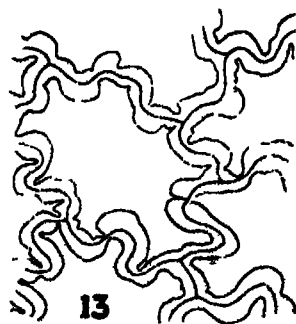
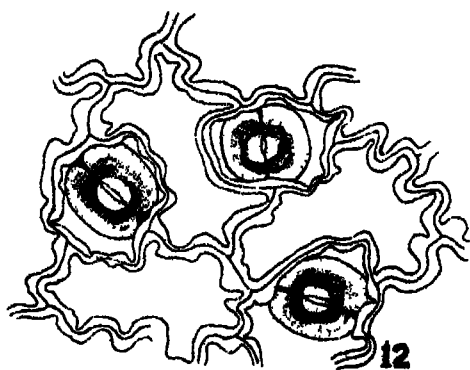
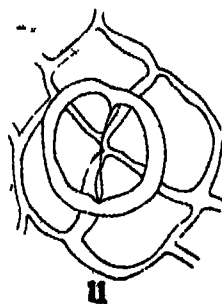
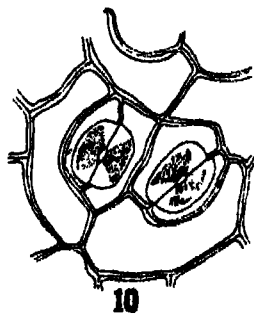
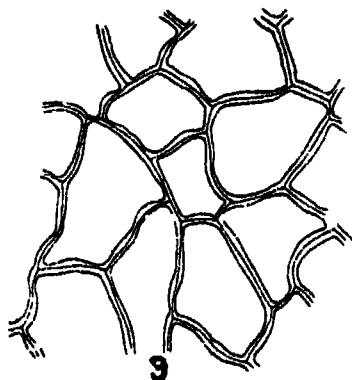
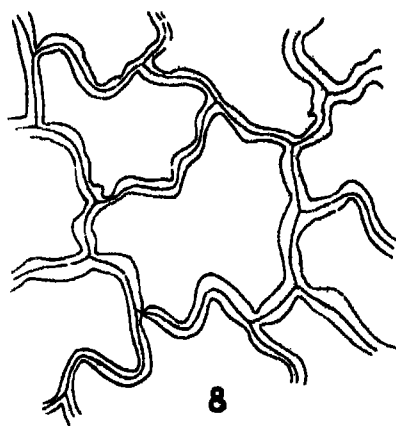
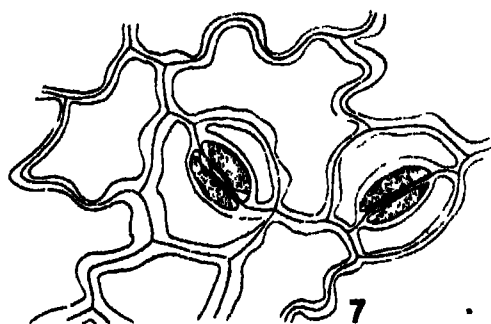
- Figs 1 & 2 *Magnolia nitida*, W W Sm (Yunnan) Lower and upper epidermis, × 400.
- Figs 3 & 4 *M. Mamyay*, King (Malayan Archipelago) Lr and up epid In the up. epid there are a large number of striations in the thickness of cell walls (not shown in drawing) which may be due to the flattening of the cuticle on the slide, so that the inward projections of the walls are folded 3, × 335, 4, × 500
- Figs 5 & 6 *M. Henryi*, Dunn (China) Lr and up epid Note the longitudinal division in one of the lateral subsidiary cells. Pits are present in the epid cells of both surfaces (not shown) 5, × 335; 6, × 400
- Figs 7 & 8 *M. obovata*, Thunb (S India). Lr and up epid × 400
- Figs. 9 - 11 *M. rostrata*, W.W.Sm (Yunnan) 9, up. epid, 10, Lr. epid. Note the longitudinal division in one of the lateral subs cells; 11, hair-base × 500.

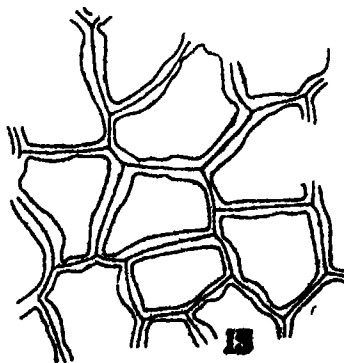
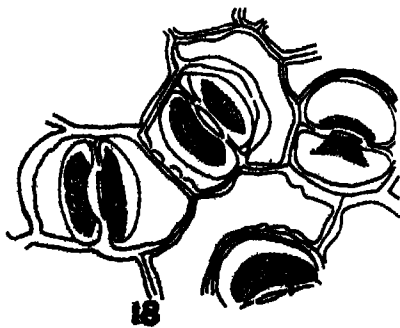
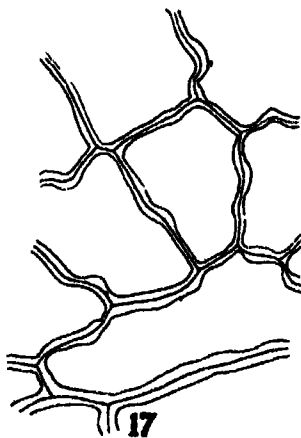
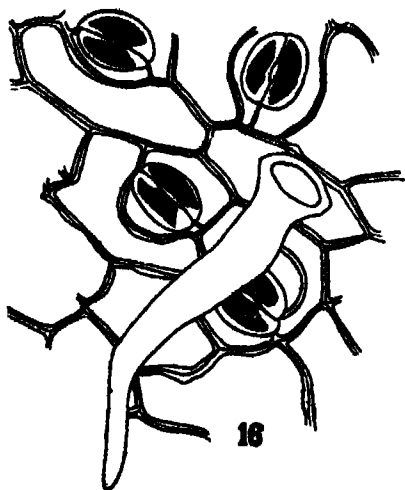
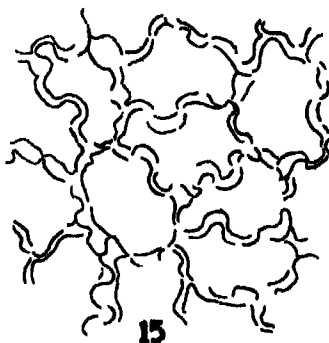
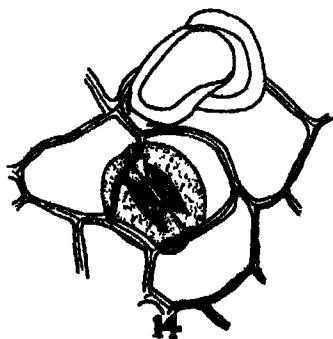
- FIGS 12 & 13 *M. Championi*, Bth (Hongkong) Lr and up, epid. Pits present on both surfaces (not shown) × 500.
- FIGS 14 & 15 *M. grandiflora* L. (Cultivated in R.B.G.) 14, Lr, epid. Note the hair-base. × 500.
- FIGS 16 & 17 *M. conspurua*, sal forma? (China) Lr and up, epid. Note the long divn in one of the lateral subs cells. × 400
- FIGS 18 & 19 *M. Delavayi*, Franch (Yunnan) Lr and up, epid. Note the long divn in one of the lateral subs cells × 500
- FIGS 20 & 21 *M. taberna*, W.W Sm (Yunnan) Lr and up epid × 500
- FIGS 22 & 23 *M. pterocarpa* (*M. sphenocarpa*) (Cultd in R.B.G.) Lr and up epid Pits present on both surfaces (not shown) × 500, 22, × 500, 23, × 325
- FIGS 24 & 25 *M. hypoleuca*, s z (Japan) Lr and up epid. × 500
- FIGS 26 & 27 *M. lobus*, Dr (Japan) Lr and up epid × 500
- FIGS 28 & 29 *Liriodendron tulipifera* (Cultd in R.B.G.) Lr and up epid. Note the stomata-free areas over the veinlets, with elongated cells. × 400
- FIGS 30 & 31 *Manghetia Hookeri*, Oubitt and Smith (Burma) Lr and up, epid × 400.
- FIGS 32 & 33 *M. insignis*, Bl var *angustifolia* (Assam) Lr and up epid. × 400
- FIGS 34 & 35 *M. angustifolia*, Hk. f and T (Burma) Lr and up epid. × 500.
- FIGS 36 & 37 *M. glauca*, Bl (Malayan Archipelago) Lr and up epid × 400
- FIGS 38 & 39 *Michelia fuscata*, Bl (Cultd in R.B.G.) Lr and up, epid × 400.
- FIGS 40 & 41 *M. manni*, King (Assam) Lr and up epid × 400
- FIGS 42 & 43 *M. Cathartii* Hk f and T (Naga Hills) Lr. and up epid × 400
- FIGS 44 & 45 *M. montana*, Bl (Assam) Lr and up epid. Note the long divn in one of the subs cells × 630
- FIGS 46 & 47 *M. krasota*, Ham (Nepal) Lr and up epid × 400
- FIGS 48 & 49 *M. punduana*, Hk f and T (Assam) Lr and up epid. × 400
- FIGS 50 & 51 *M. champaca*, L. (India and Burma) Lr and up epid. × 400
- FIGS 52 & 53 *M. nilagirica*, Zenk var *Walleri* (Peninsular India and Ceylon) Lr. and up epid. Note the long divn in one of the subs cells Pits present (not drawn) × 400.
- FIGS 54 & 55 *M. oblonga*, Wall (Assam) Lr and up epid × 400.
- FIGS 56 & 57 *M. globosa*, Hk. f and T (Sikkim) Up and lr, epid × 300.
- FIGS 58 & 59 *Talauma Candollei*, Bl (Cultd in R.B.G.), 58 and 61 up and lr, epid. 59 and 60, 1st and 2nd divns. in the development of stoma according to the syndetocheile type. 58 & 61, × 400; 59 & 60, × 630.
- FIGS 62 & 63 *T. spongiaripa*, King (Upper Burma). Up and lr, epid. Pits present (not drawn). × 500.
- FIGS 64 & 65 *T. Forbesii*, King (Sumatra). Lr. and up epid. Note the long. divn. in one of the subs cells × 500.
- FIGS 66 & 67 *T. Maingayi*, King (Malaya) Lr and up epid. Pits present. × 500.
- FIGS 68 & 69 *T. villosa*, Miq. (*T. lanigera*, Hk. f. and T.) (Malayan Peninsula.) Lr. and up epid. Pits distinct on both surfaces. × 400.

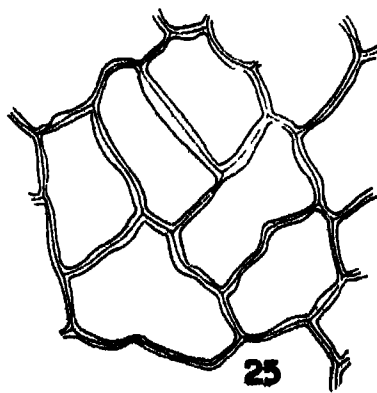
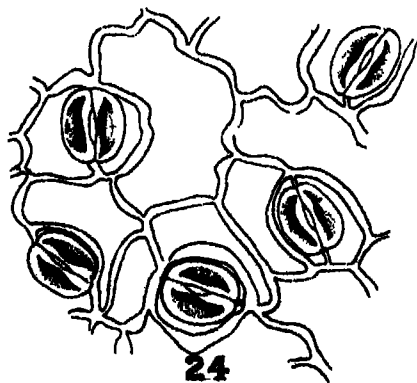
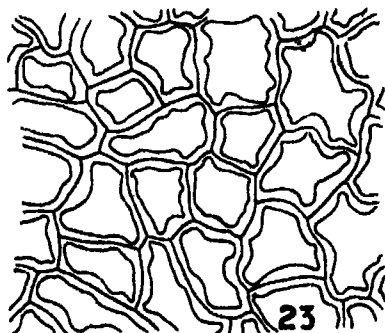
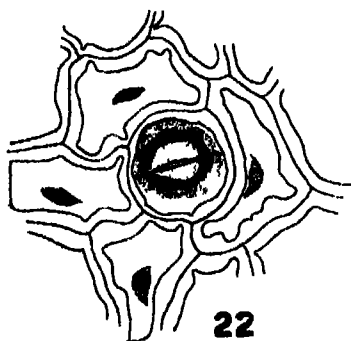
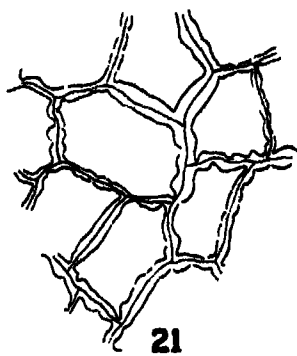
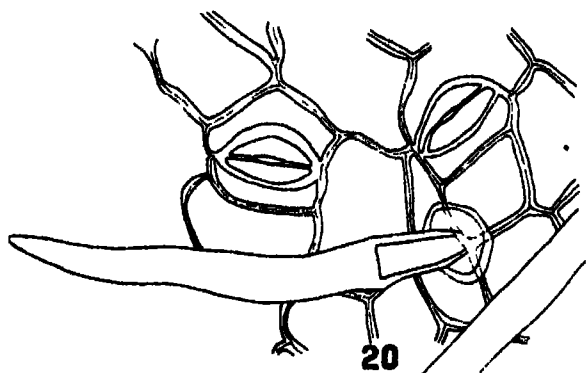
- FIGS. 70 & 71 *T. Küntleri*, King (Kedah Peak) Lr and up epid $\times 400$.
- FIGS. 72 & 73 *T. Hodgsoni*, Hk f and T (Sikkim Himalaya) Lr and up epid $\times 500$
- FIGS 74 & 75 *T. mutabilis*, Bl (Malayan Archipelago) Up. and lr epid. Note the long. divn. in one of the subs cells Pits on both surfaces (not shown). $\times 400$
- FIGS 76 & 77 *T. andamanica*, King (Andamans) Lr and up epid Note the long divn in one of the subs cells Pits on both surfaces (not shown) $\times 500$
- FIGS 78 & 79 *Aromadendron elegans*, Bl Lr and up epid $\times 630$.
- FIGS 80 & 81 *Drimys winteri*, Lr and up epid Note the long divns in both the subs. cells $\times 300$
- FIGS 82 & 83 *D. aromatica* Lr and up epid Note the long divns in both the subs cells $\times 300$
- FIGS 84 & 85 *Illicium Griffithii*, Hk f. and T (Assam). Lr and up epid Pits present on both surfaces (not shown in lr epid) $\times 400$
- FIGS 86 & 87 *I. cambodianum*, Stance (Malayan Peninsula). Lr and up epid $\times 400$
- FIGS 88 & 89 *I. evenum*, King (Malayan Peninsula) Lr and up epid There is seen a tendency towards long. orientation of stomata parallel to the length of the leaf Note the variation in the number of surrounding cells (2 to 5) Pits present. $\times 400$
- FIGS 90 & 91 *I. Simonsen* (Manipur) Lr and up epid Note cutin striations shown as dotted lines $\times 100$
- FIGS 92 & 93 *I. manipurensis*, Watt Ms (Assam) Lr and up epid Pits present $\times 400$.
- FIGS 94 & 95 *Schizandra propinqua*, Hk f and T (Nepal) Lr and up epid This also shows cutin striations (not shown) $\times 400$
- FIGS 96 & 97 *S. axillaris*, Hk f and T (Assam). Lr and up epid cutin striations present (not shown) Note the long divns in one of the subs cells and tr divns in the other 96, $\times 335$; 97, $\times 400$.
- FIGS. 98 & 99 *S. elongata*, Hk. f and T (Himalaya) Lr and up epid Note the long divn in one of the subs cells and two tr divns in the other
- FIGS 100 & 101 *Kadsura cauliflora*, Bl (Penang) Lr and up. epid Note the tr divn. in one of the subs cells $\times 400$
- FIGS. 102 & 103 *K. Roxburghiana*, Arn (Burma) Lr and up. epid. Note the tr divns in both the subs cells Note also the hair-base on up epid. $\times 400$
- FIGS. 104 & 105 *K. scandens*, Bl. Lr and up epid Note the tr. divn in both the subs cells Note also the hair-base (present on both surfaces). 104, $\times 335$, 105, $\times 400$.
- FIGS. 106 & 107 *K. lanceolata*, King (Malaya). Lr and up epid Note the cutin striations $\times 400$
- FIGS. 108 & 109 *Trochodendron aralioides*, Veitch Lr and up epid. $\times 630$
- FIGS. 110 & 111 *Eupalea Franchetii*, Chénault. Lr. and up epid. Note the varying numbers of surrounding cells. This belongs to the haplocheile type. Note the differences in sizes of the stomata. $\times 325$.

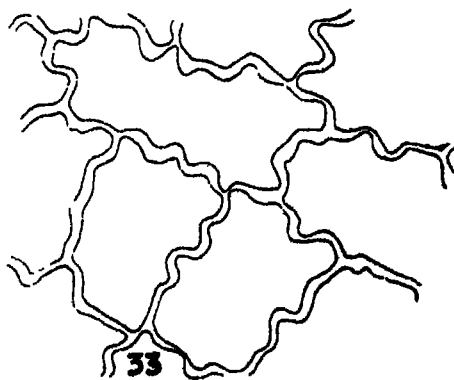
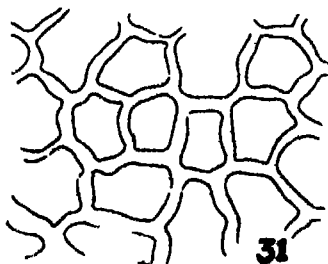
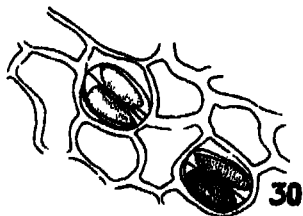
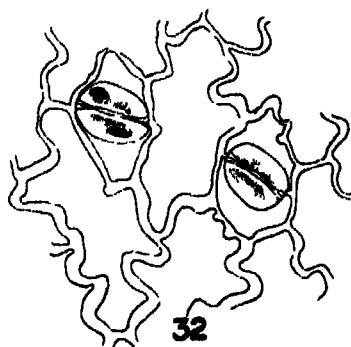
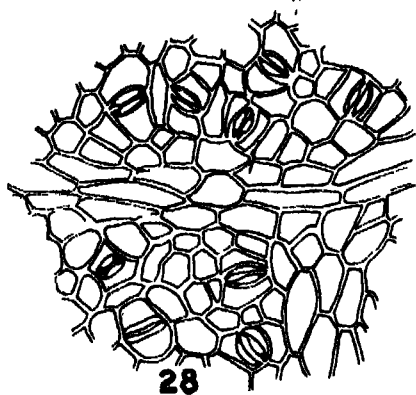
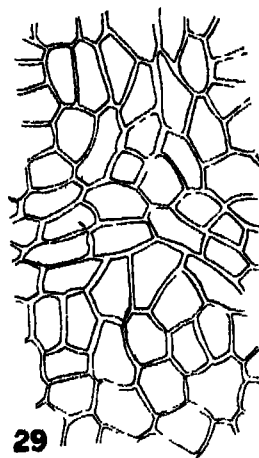
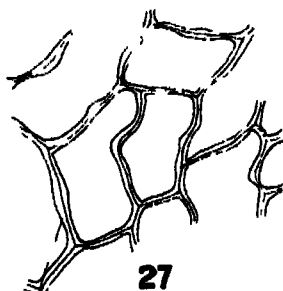
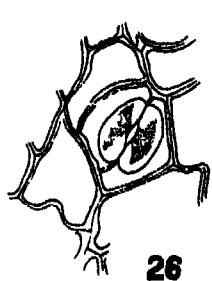
- FIGS. 112 & 113 *Tetracentron sinense* Lr and up epid. Note the tr. divns. in both the sub cells. Cutin striations are present (not shown). $\times 800$.
- FIGS 114 & 115 *Cercodiphyllum japonicum* Lr and up epid. Note that there are seven cells surrounding the stoma. This belongs to the haplocheile type. $\times 712$.
- FIG 116 *C japonicum* Median tr section of stoma. Note the thickenings of the guard cells and the cutin projections forming the outer vestibule (a Bennettitalean character) $\times 712$
- FIG 117 *C japonicum* L S of guard cell $\times 1425$.
- FIG. 118 *C japonicum*. T S. of guard cells at the polar end $\times 630$.
- FIG 119 *C japonicum* T S. between the pole and median position of stoma. $\times 630$
- FIG. 120 *C japonicum*. Section of the upper epidermis showing the pits extending part way into the cuticle
- FIGS 121 & 122 *Drumys aromatica* L S and T S (one half) of stoma $\times 630$.
- FIGS 123 & 124 *Michelia champaca*, L (Mysore) Two drawings of the tr section of stoma drawn from a hand-section by focussing at different levels, 0.6 mm apart $\times 712$
- FIG 125 *M champaca*, L. Drawn as in the previous case. Difference between I and II— 1 mm, between II and III— 0.8 mm $\times 712$.
- FIG 126 *M. champaca*, L L S of guard cell $\times 712$.
- FIGS 127 & 128 *Liriodendron tulipifera* L S. and T.S (oblique) of stoma. $\times 1425$

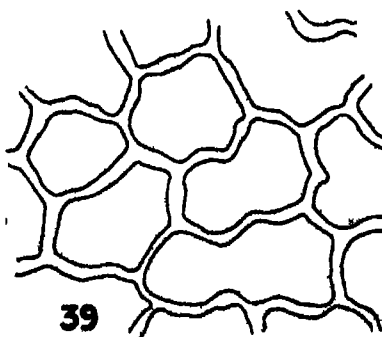
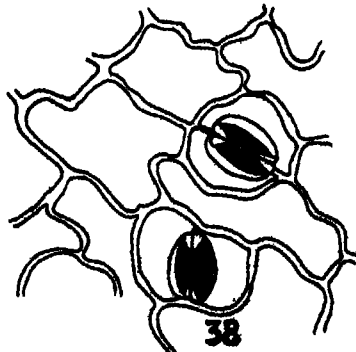
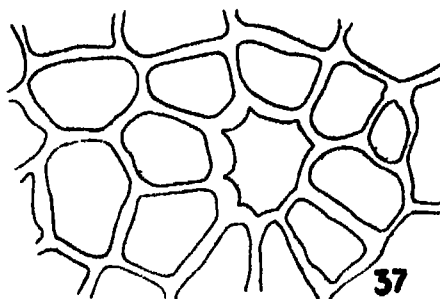
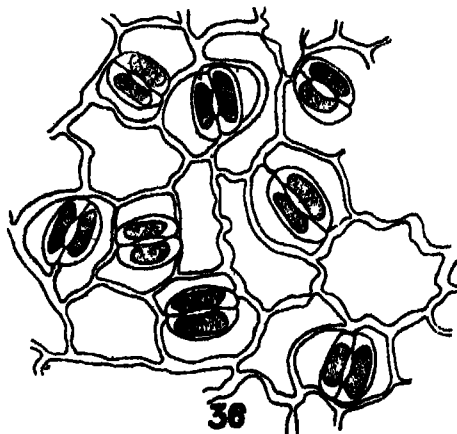
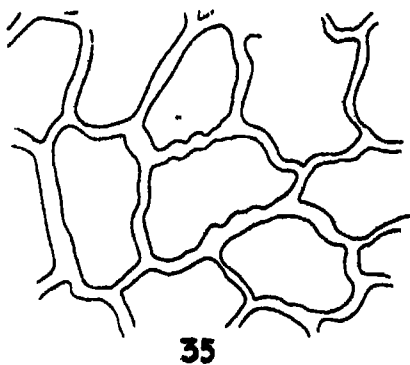
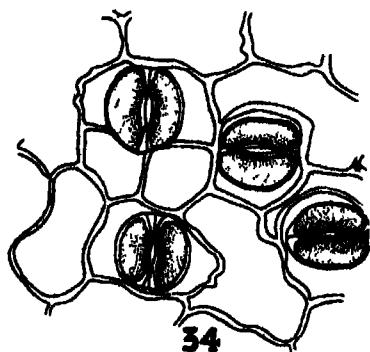


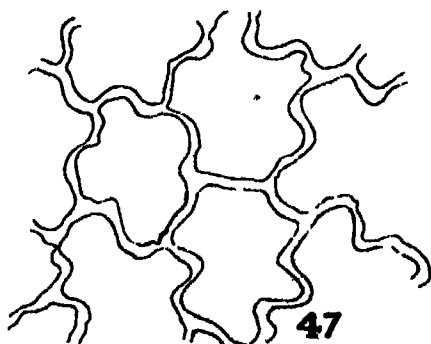
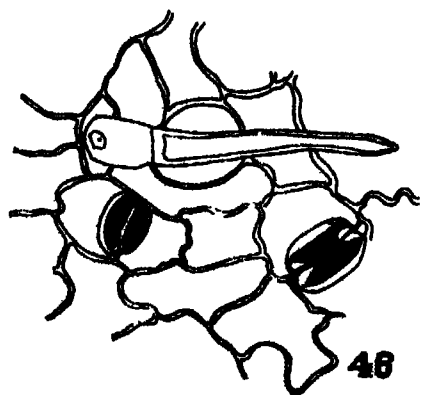
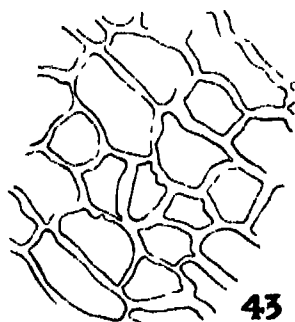
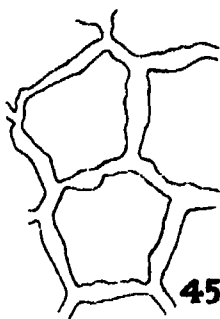
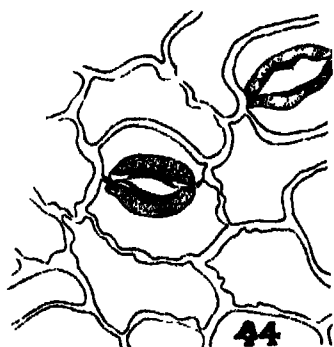
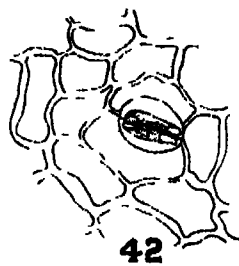
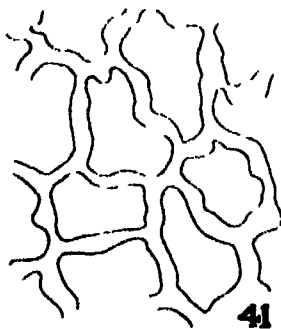


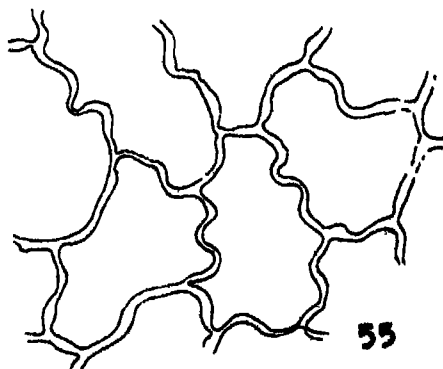
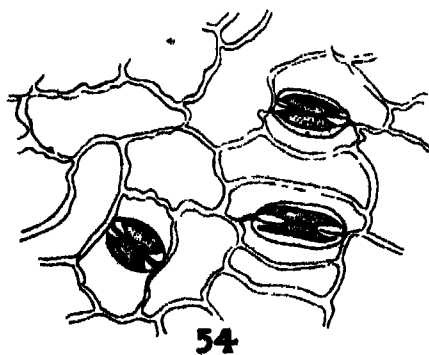
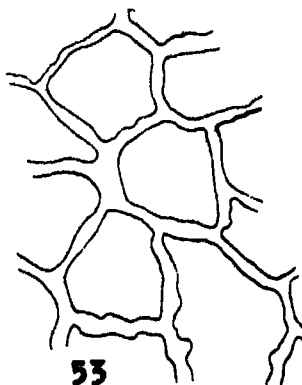
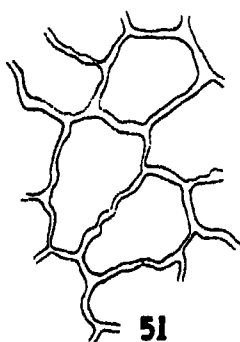
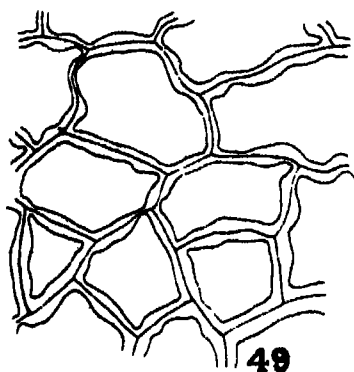
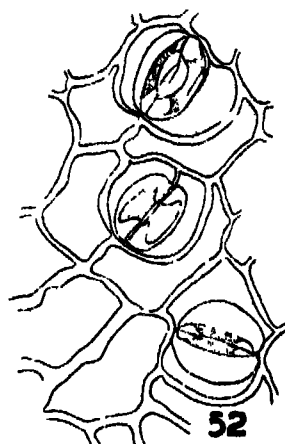
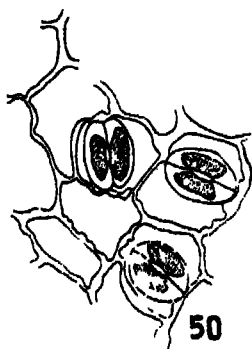
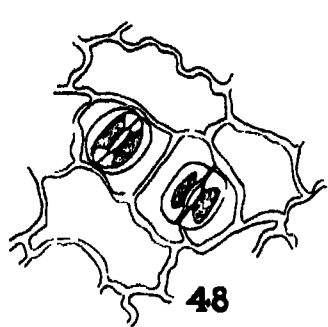


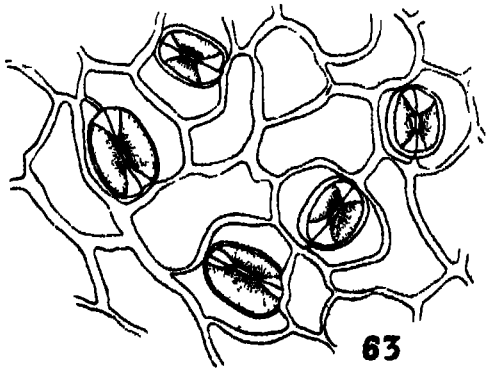
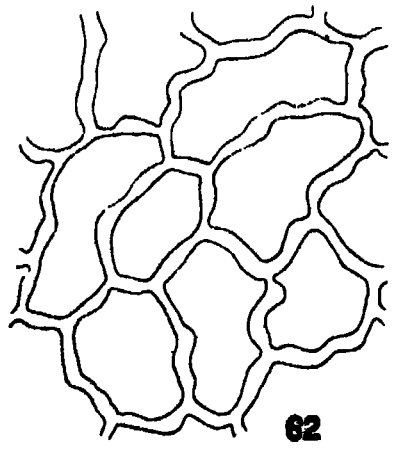
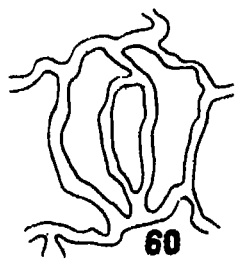
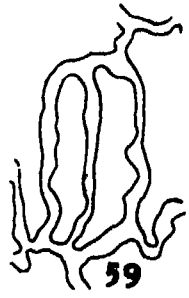
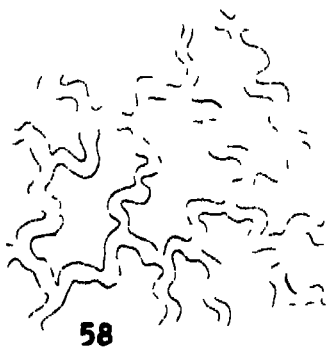
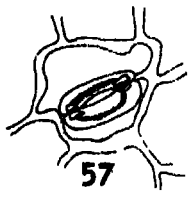
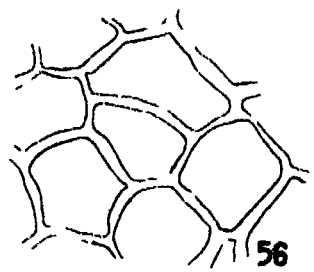


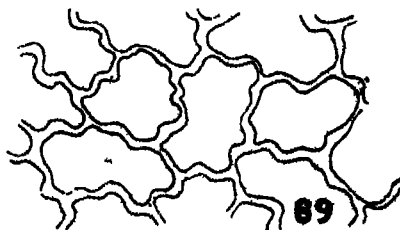
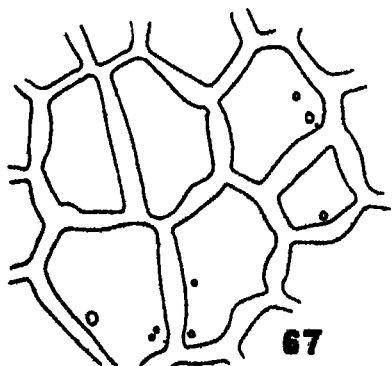
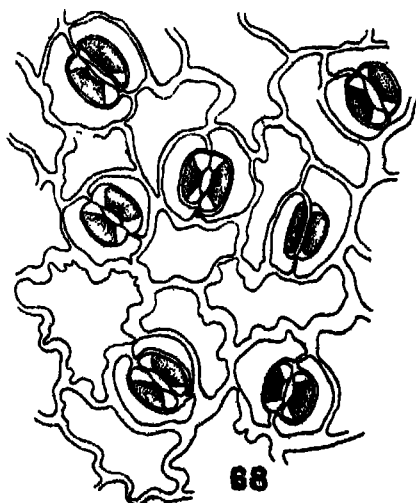
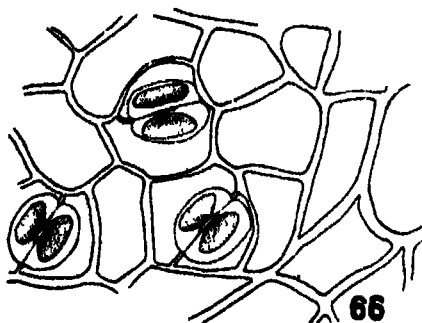
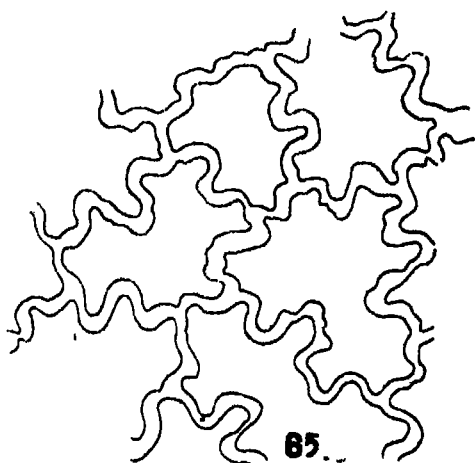
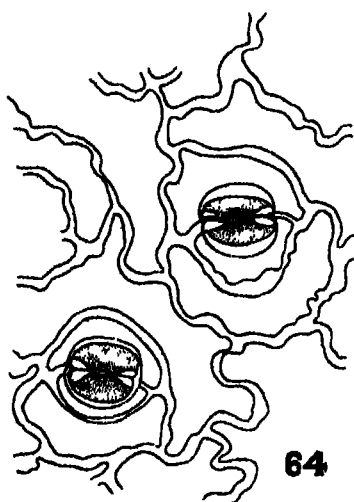


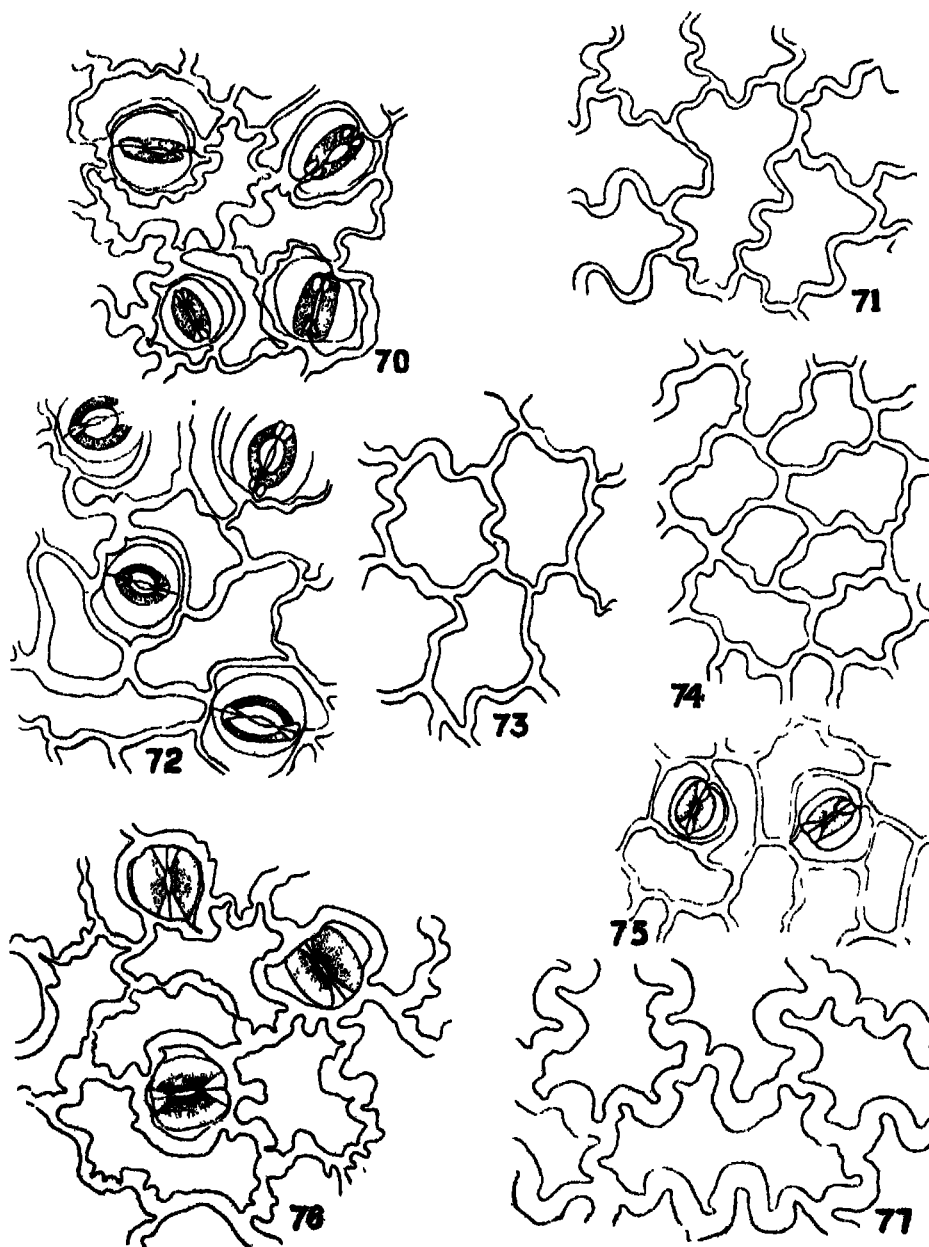


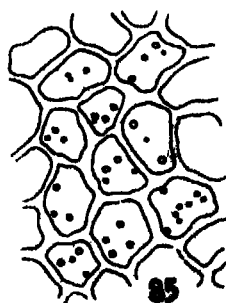
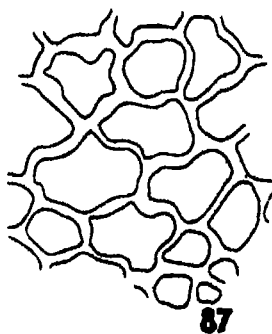
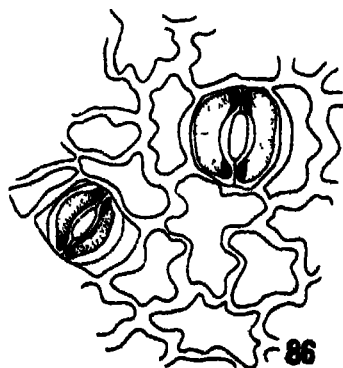
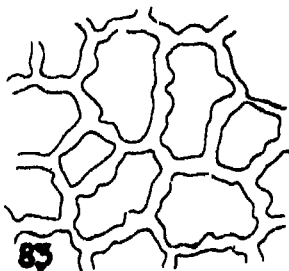
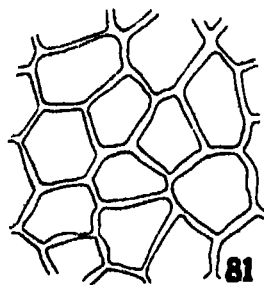
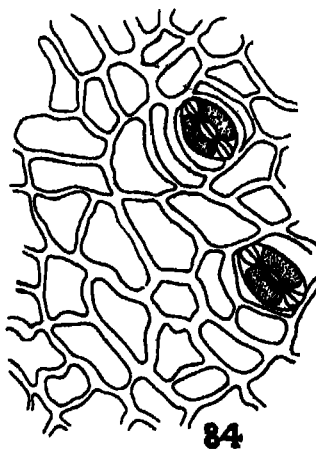
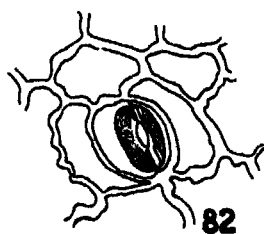
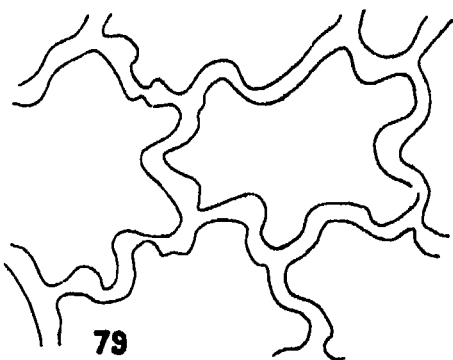
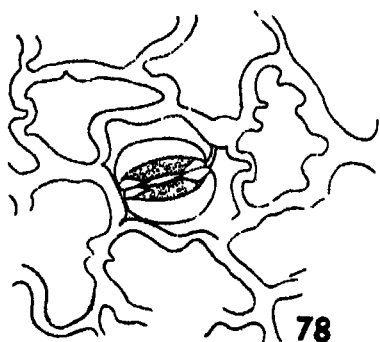


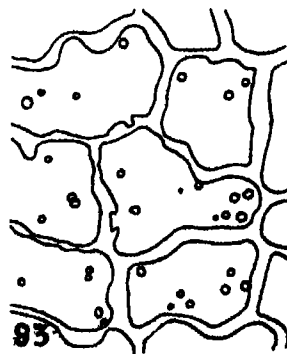
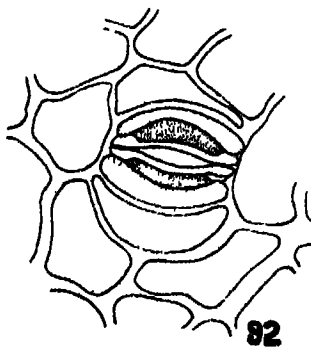
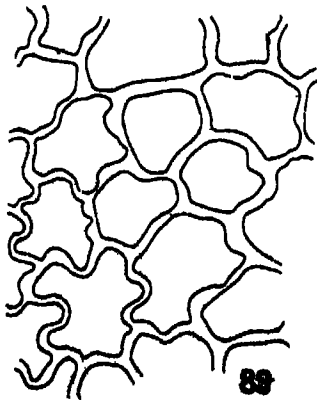
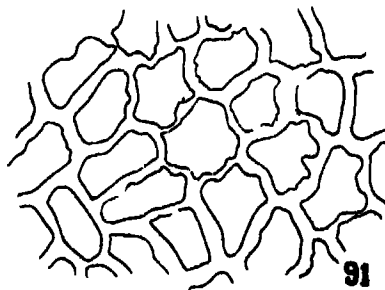
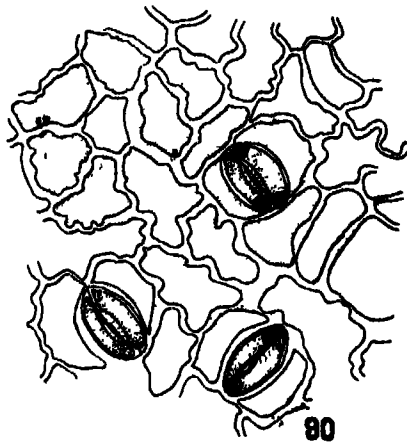
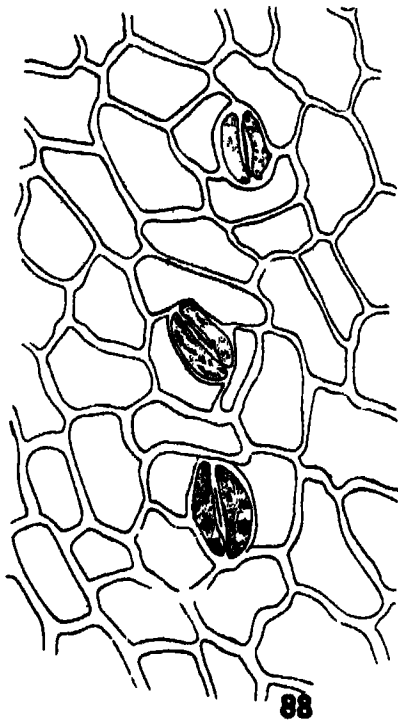


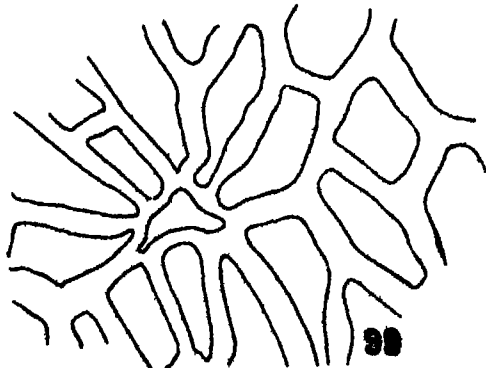
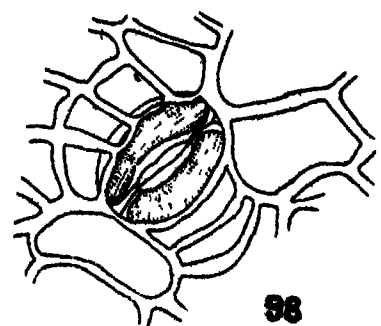
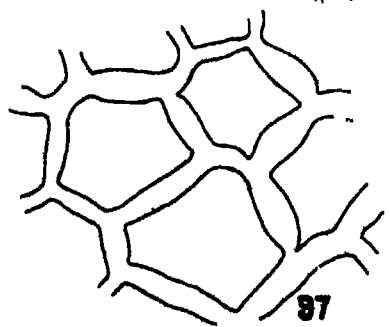
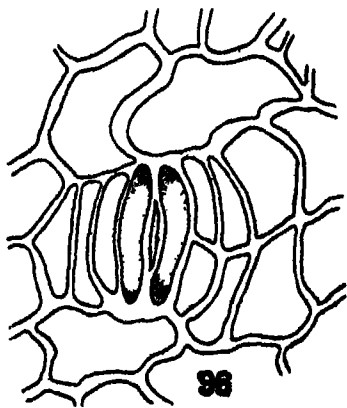
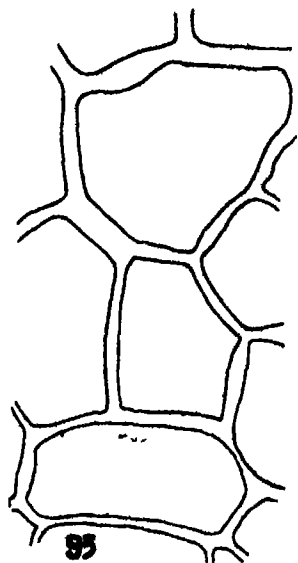
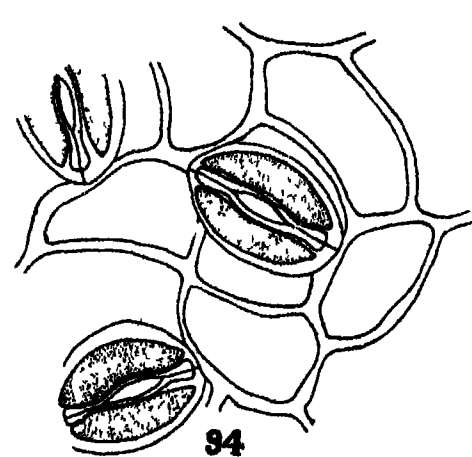


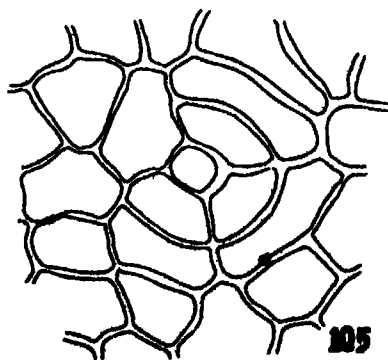
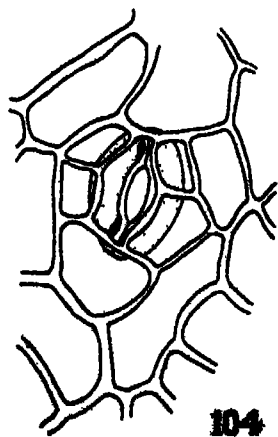
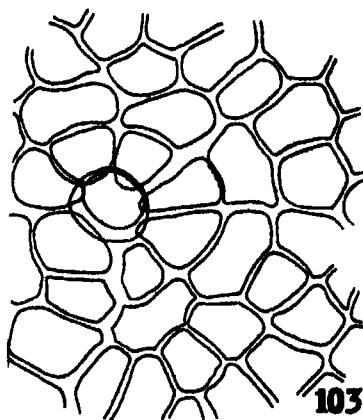
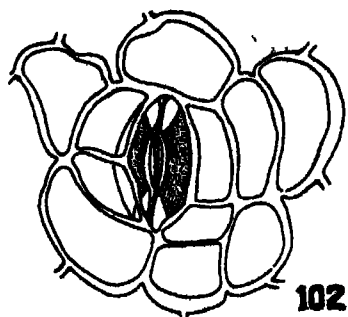
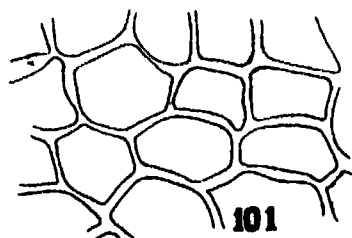
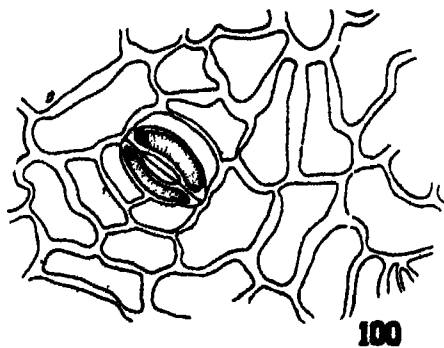


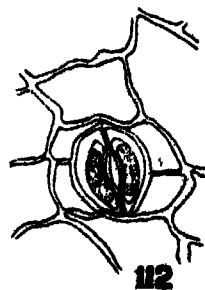
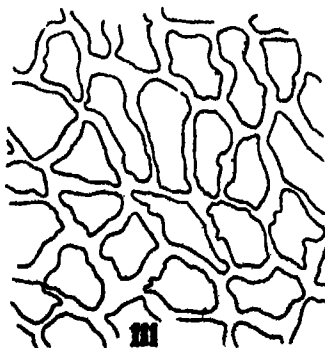
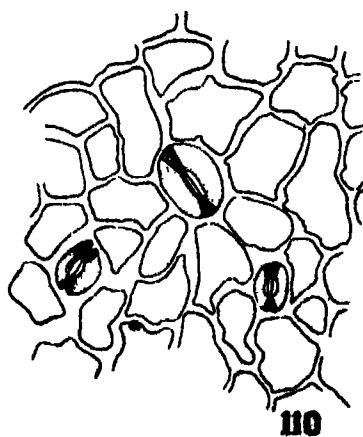
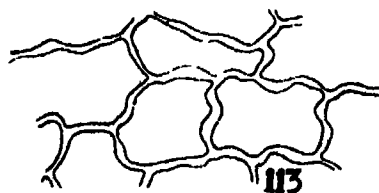
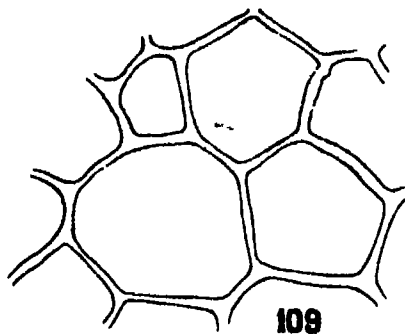
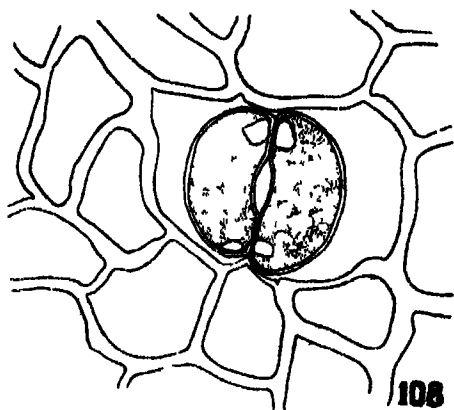
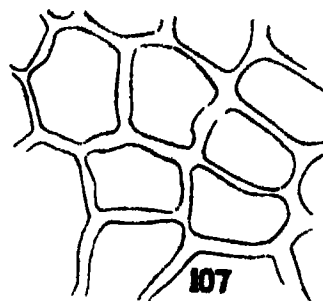
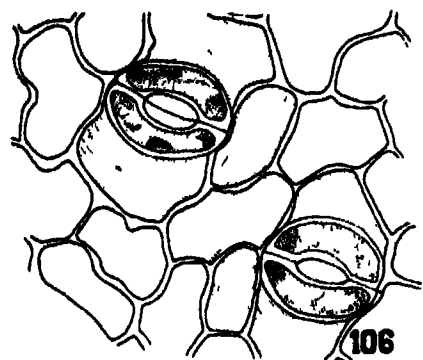


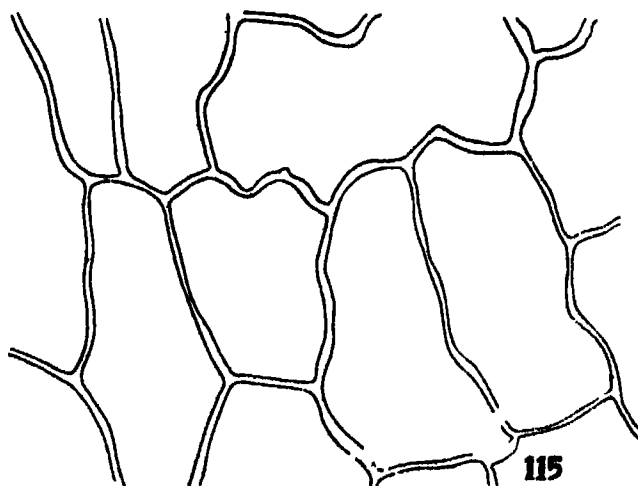
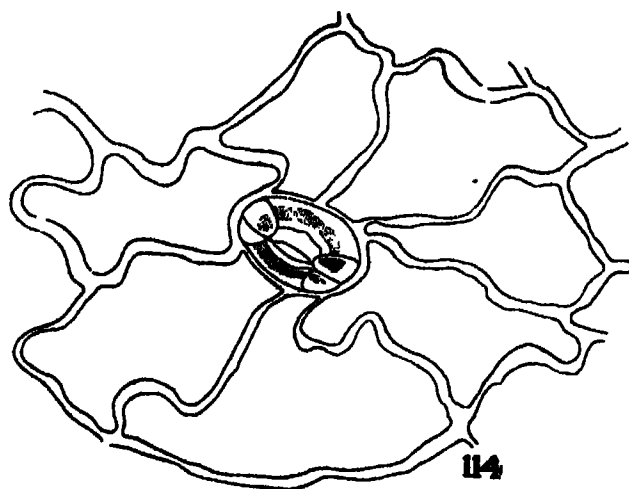


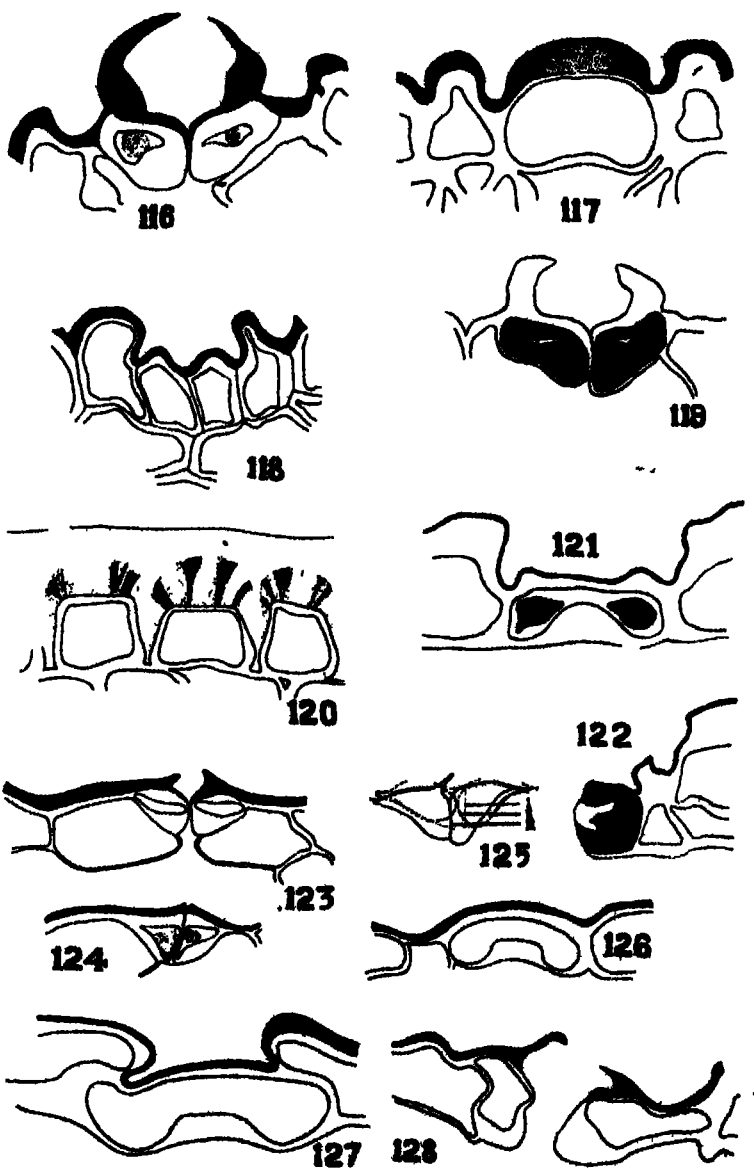












A STUDY OF THE EFFECT OF DIFFERENT TYPES OF RATIONS ON THE QUALITY OF MILK, MILK YIELD AND THE GENERAL CONDITION OF MILCH BUFFALOES

BY D V BAL

Agricultural Chemist to Government, C P and Berar

AND

S K MISRA

Lecturer in Animal Husbandry & Dairying, College of Agriculture, Nagpur

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I Introduction

BUFFALOES are the chief milch animals in the Central Provinces and Berar. Milch buffaloes are generally fed with roughages of different types according to the conditions obtaining in different tracts and with concentrates in which cotton seed is, wherever possible, one of the important constituents. Amongst the oil-cakes linseed-cake finds favour with the *Gaohies* although it is generally more costly than groundnut cake. This may be due to the fact that the former has a better keeping quality than the latter. Amongst the local *Gaohies* and cultivators who maintain buffaloes for the purpose of milk production, there is a belief that if the animals are fed with oil-cakes instead of cotton seed, there is a deterioration in the quality of the milk although the milk yield may remain unaffected.

As cotton seed is sometimes comparatively dearer in the market than other food-stuffs which are capable of supplying unit per unit the same quantities of proteins and carbohydrates, and in view of the fact that large quantities of groundnut cake and *juar* are produced in this Province, it was considered desirable to find out if these two materials could be suitably substituted for other comparatively costly substances which are used as concentrated food-stuffs for milch cattle.

Some feeding experiments were therefore conducted at the Agricultural College Dairy, Nagpur, to test the effect of two different rations on the milch cattle (buffaloes), one consisting of groundnut cake in combination with *twr chums* and *juar* meal and the other consisting of cotton seed in combination with linseed-cake and *twr chums*. The results obtained are recorded in this paper.

II Experimental

The following standard rations found to be adequate for the production of 100 lb of milk were employed in the feeding experiments referred to in this paper, and the actual quantities of the ration given per animal were regulated according to the individual milk yield

Ration A

Kind of ration	Quantity fed	Digestible crude protein	Total digestible nutrients	Cost of ration according to prevalent market rates
	lbs	lbs	lbs	R A P
<i>Tur chuni</i>	20	3 08	15 28	} 1 13 2
Groundnut cake	10	4 20	7 88	
<i>Juar meal</i>	40	2 58	27 81	
TOTAL	70	9 86	50 97	-

Ration B

<i>Tur chuni</i>	20	3 08	15 28	} 2 4 0
Linseed cake	10	2 60	7 90	
Cotton seed	35	4 27	28 10	
TOTAL	65	9 95	51 28	

Two groups each consisting of 3 buffaloes, were selected for the purpose of the feeding experiments. One group was fed with ration "A" and the other with ration "B" referred to above, in addition to the usual roughages available on the farm during the various seasons. These groups of animals are designated as "Group A" and "Group B" respectively in the paper.

At the commencement of the experiment, the animals selected were in different stages of lactation and they were therefore kept under observation till they reached the same respective stages of lactation subsequently. This procedure enabled us to study the behaviour of the animals for a period equivalent to one full lactation.

III Effect of the Two Rations on the General Health of the Animals

In order to determine the effect of the two rations on the health of the animals, a record of their monthly weights and the weights of the calves at the time of birth were maintained and the data obtained in this connection are given in Table I.

TABLE I
Monthly live-weights of the Animals and the weights of the Calves at the time of birth in lb
(Monthly live-weights represent the averages of weights recorded on 3 consecutive days in each case)

Names of animals	Initial	After 1 month	After 2 months	After 3 months	After 4 months	After 5 months	After 6 months	After 7 months	After 8 months	After 9 months	After 10 months	After 11 months	After 12 months	After 13 months	After 14 months	After 15 months	After 16 months	After 17 months	After 18 months	After 19 months	After 20 months
	May 1931	June	July	Aug	Sept	Oct	Nov	Dec	Jan 1932	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan 1933
Group "A"																					
Paithan	1145	1092	1099	1115	1137	1105	1143	1196	1189	1228	1247	1287	1321	1323	1351	1306	(a) 1216	1241			
Mankoo	1183	1150	1168	1219	1245	1276	1355	1407	1403	1260	1269	1279	1316	1301	1311	1260	1233				
Madhuri	836	827	864	889	881	891	896	(c) 944	831	847	833	877	885	890	873	830	885	923			
Group "B"																					
Gulbi	1080	1033	1064	1099	1064	1049	1080	1099	1124	1098	1124	1122	1148	1184	1171	(d) 1012	1064	1080			
Madan	1070	1025	1033	1047	1033	1024	1064	1091	1120	1151	1163	1170	1165	1234	1260	1186	1167	1264	1335	(e) 1220	1233
Begun	1032	1036	1087	1185	1148	1165	1204	1284	(f) 1158	1177	1129	1140	1188	1206	1209	1184	1209	1269	1372	(g) 1224	

(a) Calved on 21st August 1932, weight of the calf = 70

(b) " " 13th January 1932 " = 85

(c) " " 24th November 1931 " = 64

(d) " " 18th July 1932 " = 65

(e) " " 23rd November 1932 " = 68

(f) " " 18th December 1931 " = 60

(g) " " 10th November 1932 " = 70

Figures given in Table I show clearly that all the animals either maintained their weights or showed some increase during the experimental period. Observations on the general conditions of the animals made from time to time also showed that animals from both the groups maintained, on the whole, perfect health and excellent condition throughout the experiment. The animals however suffered the usual loss in weight due to calving, but this loss was soon made up and the animals again became normal in due course.

Weights of the calves at birth from the two groups were also found to be normal, the actual weights recorded being 60 to 85 lb per head.

A combination of cotton seed and linseed-cake in the ration fed to buffaloes does not therefore show any particular advantage over groundnut cake and *juar* meal.

IV *Effect of the Two Rations on the Milk Yield*

Milk yields of the various animals, obtained during the experimental period, together with those for the previous lactation in each case are given in Table II.

TABLE II
Average daily milk yields and the total yields of milk of individual animals

Names of animals	Milk yield during the experimental period equivalent to one lactation				Milk yield of the previous full lactation, prior to the experimental period		
	No. of individual lactations of the animals while under experiment	Total milk yield in lbs	No. of days in milk	Average milk yield per day in lbs	Total milk yield in lbs	No. of days in milk	Average milk yield per day in lbs
<i>Group "A"</i>							
Palthan	11 & 12	4,013	349	11.50	3,583	301	11.90
Mankoo	5 & 6	4,760	311	15.30	5,515	356	15.49
Madhuri	2 & 3	3,840	377	10.24	3,171	375	8.46
<i>Group "B"</i>							
Gulbi	11 & 12	3,931	376	10.46	3,636	371	9.80
Malan	1 & 2	6,796	563	12.06	6,291	563	11.17
Begum	2 & 3	3,429	299	11.47	2,902	314	8.92

TABLE III—(Contd.)
Monthly averages of the analytical figures of milk samples of buffaloes from "B Group"

Names of individual animals	Samples from individual animals			Composite samples					
	Malan	Gulbi	Begum						
	Fat %	Fat %	Fat %	Sp Gravity	Total solids %	Fat %	Ash %	P ₂ O ₅ %	CaO %
Initial at start	8.83	8.73	10.48	1.029	18.61	9.40	0.817	0.276	0.290
1st month	8.58	7.93	10.87	1.031	18.25	9.18	0.826	0.267	0.284
2nd "	8.44	7.81	10.94	1.032	18.38	8.69	0.845	0.290	0.266
3rd "	8.14	7.79	↑	1.032	17.92	8.01	0.811	0.269	0.249
4th "	8.02	8.21	animal	1.031	18.08	8.13	0.786	0.269	0.262
5th "	8.26	8.17	dry	1.031	18.25	8.39	0.806	0.279	0.270
6th "	8.40	9.03	↓	1.031	18.61	8.72	0.848	0.280	0.262
7th "	9.32	8.99	↓	1.030	19.56	9.23	0.900	0.304	0.269
8th "	9.56	9.41	7.02	1.030	19.80	9.59	0.802	0.311	0.261
9th "	10.03	9.92	6.84	1.030	18.36	8.71	0.835	0.297	0.284
10th "	9.89	9.90	7.47	1.030	18.22	8.70	0.806	0.281	0.280
11th "	9.51	9.16	7.65	1.030	18.12	8.29	0.795	0.278	0.269
12th "	9.57	↑	7.88	1.030	18.64	8.19	0.813	0.275	0.261
13th "	9.68	animal	8.11	1.030	18.60	8.70	0.799	0.283	0.284
14th "	9.42	dry	8.41	1.030	18.60	8.68	0.810	0.300	0.264
15th "	9.31	↓	9.30	1.031	19.07	8.87	0.833	0.294	0.286
16th "	↑	6.14	9.41	1.030	18.72	8.61	0.873	0.325	0.317
17th "	animal	6.32	9.41	1.030	16.75	6.74	0.795	0.268	0.277
18th "	dry	7.08	↓	1.030	16.80	7.07	0.748	0.243	0.264
19th "	↓	↓	↓	1.032	16.75	6.52	0.789	0.267	0.276
20th "	6.43	↓	↓	1.030	16.84	7.20	0.734	0.243	0.252
21st "	7.02	↓	↓	1.030	17.71	8.03	0.728	0.243	0.258
22nd "	8.03	↓	↓	1.030	17.71	8.05	0.707	0.241	0.242
23rd "	7.73	↓	↓	1.028	16.99	7.99	0.700	0.243	0.230
24th "	8.24	↓	↓	1.030	17.31	6.19	0.703	0.252	0.240
25th "	8.00	↓	↓	1.030	17.31	6.19	0.703	0.252	0.240
26th "	8.00	↓	↓	1.030	17.31	6.19	0.703	0.252	0.240

These results indicate clearly that the average daily milk yield of the animals, which were either in their fifth or subsequent lactations, is neither adversely affected nor particularly improved by the exclusion or inclusion of cotton seed with linseed-cake in thier daily diet. In the case of the young animals from both the groups there was the usual increase in the daily yield of milk which normally takes place till the animal reaches the fourth or fifth lactation.

V Effect of the Two Rations on the Composition of Milk

Fat and mineral contents of the individual and average samples of milk collected from the animals belonging to each group were determined twice a week throughout the experimental period and the results obtained are given in Table III. Separate analyses of morning and evening samples of milk were carried out but as the figures obtained did not show any marked variation, average results only are given in the table. It was found that, except for occasional changes usually associated with the advancement of the lactation period or with variations in health, there were no characteristic changes in the percentage of fat in milk as a result of the difference in the two diets employed. There was no appreciable variation in the mineral composition of the milk in respect of phosphoric acid (P_2O_5) and calcium (CaO).

VI Effect of the Two Rations on the Quality of the Butter Fat

Composite samples of milk from the animals belonging to both "Groups" were collected once before the commencement of the experiment and on three occasions during the course of the experiment for the determination of the following fat constants —(1) melting point, (2) Reichert-Meissl value, (3) Polenske value. Results obtained are given in Table IV.

TABLE IV

Dates of sampling	Melting point		Reichert Meissl values		Polenske values	
	A Group	B Group	A Group	B Group	A Group	B Group
5-5-31 (Initial)	36.4	35.50	35.12	35.59	1.436	1.372
3-5-32	35.9	37.30	37.55	29.66	1.038	1.163
20-5-32	35.7	37.50	37.59	29.84	1.501	0.877
27-6-32	35.5	37.90	39.68	29.77	1.704	1.024

The results given above show that the diet containing cotton seed remarkably affects the physical characteristics of the butter fat by making it harder as seen from the higher melting point of the butter fat of animals of group " B " than that of the butter fat obtained from the animals of group " A "

The amount of steam-volatile, water-soluble and water-insoluble fatty acids as judged by the Reichert-Meissl and Polenske values respectively, is however, strikingly lower in the samples of butter obtained from animals receiving cotton seed than that present in the samples of butter obtained from those receiving *juar* meal and groundnut cake

This observation clearly indicates that samples of butter and *ghee* available in the local markets should ordinarily give a much higher Reichert-Meissl value than the minimum of 19.0 fixed under the C P Prevention of Adulteration Act of 1919, as the milch animals providing the bulk of the marketable *ghee* are fed largely with concentrates resembling those included under diet " A " in this investigation, along with a small proportion of cotton seed.

VII Summary

(1) Amongst the local *Gaohes* and cultivators there is a belief that if milch animals are fed with oil-cakes instead of cotton seed, there is a deterioration in the quality of the milk although the milk yield may remain unaffected.

(2) Some feeding experiments were therefore conducted to test the effect of two different types of rations on the milch cattle (buffaloes), one consisting of groundnut cake in combination with *tur chuni* and *juar* meal and the other consisting of cotton seed in combination with linseed-cake and *tur chuni*

(3) Results obtained show the following :—

- (a) Inclusion of cotton seed in the ration fed to which buffalo does not show any particular advantage over a ration consisting of groundnut cake and *juar* meal, in so far as the general health and individual weights of the animals, and of the calves at birth, are concerned
- (b) Average daily milk yields of the various animals were neither adversely affected nor particularly improved by the exclusion or inclusion of cotton seed in their daily diet and no characteristic variations in the percentage of fat in the milk were observed. These results are significant as they definitely show that the quality

(commonly understood as the percentage of fat) of the milk is not in any way lowered by the exclusion of cotton seed from the diet

- (c) Cotton seed, however, significantly affects the characteristics of the butter fat by increasing its melting point, and by lowering the proportion of the volatile water-soluble and insoluble fatty acids contained in it

(4) These results further establish the important fact that market samples of genuine butter and *ghee* should ordinarily give a much higher Reichert-Meissl value than the minimum of 19.0 fixed under the C P Prevention of Adulteration Act of 1919, and in this respect they therefore confirm the results previously obtained by Plymen ¹

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THE PROTEINS OF BAJRI (*PENNISETUM TYPHOIDEUM*)

BY V S ABHYANKAR, W V KOTASTANE

AND

N NARAYANA

(From the College of Agriculture, Poona)

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Bajri or the spiked millet stands second to *Jowar* among the grain crops of the Bombay Presidency, and is cultivated over an area of nearly four million acres, which is 13 per cent of the gross cropped area of the Presidency. It stands fourth among the food crops of India, nearly 16 million acres being under this crop. It is entirely a rain crop, occupying the lighter descriptions of soil in all districts of moderate rainfall. It is almost always a mixed crop and as such may be grown on the same land continuously. But rotation also is frequent. The subordinate crops with *Bajri* are usually *Tur* (*Cajanus indicus*), *Udid* (*Phaseolus radiatus*) and other pulses.

Bajri is the staple diet of a large mass of people of the Presidency. The flour made into cakes or bread with or without buttermilk is considered more nutritious than rice. Narayanamurty and Ramaswamy Iyer (1931) have described an alcohol-soluble protein from this cereal. In the present paper a more complete study of all the proteins of *Bajri* is described.

Bajri flour has the following composition —

TABLE I
Proximate analysis of Bajri flour

	Per cent.
Moisture .	9.10
Ether extract . .	5.92
Proteins	9.80
Carbohydrates (by difference)	71.04
Crude fibre .	1.06
Ash	2.28

Bajri flour is rich in ether extract and contains nearly 10 per cent of proteins

Preliminary extraction experiments showed that the most suitable concentrations of sodium chloride and alcohol were 7 per cent and 75 per cent respectively. The nitrogen extracted by the various solvents is given below —

TABLE II

Solvent	Temperature °C	Nitrogen extracted in per cent of total nitrogen
Distilled water	28	12.8
7 Per cent sodium chloride	28	20.9
75 Per cent alcohol	28	35.1
75 Per cent alcohol	65	40.6

Thus 7 per cent sodium chloride solution and warm alcohol used consecutively extract nearly 60 per cent of the total nitrogen of the flour. After extracting consecutively with these two solvents the residue was treated with dilute caustic soda solution (0.01 N). Filtration was extremely difficult and a further 10 per cent of nitrogen was extracted. Therefore, by repeated extractions with these three solvents all the nitrogen in *Bajri* may perhaps be accounted for. *Bajri* therefore contains two-fifths of its proteins as a prolamin and one-fifth as a globulin.

Preparation of the prolamin.—*Bajri* flour was extracted with ether and the residue was treated with 75 per cent alcohol in the proportion of 1 to 3 and vigorously stirred for two hours. It was then filtered through a Buchner and the extract concentrated under reduced pressure till a turbidity appeared. The concentrated extract was then poured into a large volume of distilled water with constant stirring. This resulted in a milky emulsion to which was added 20 per cent sodium chloride solution till the precipitate began to appear. On being allowed to stand overnight the prolamin settled to the bottom. The supernatant liquid was siphoned off and the prolamin washed several times by grinding with distilled water. It was then pressed between filter papers, partially dried and finally extracted with ether.

Preparation of the globulins.—One kilo of *Bajri* flour was treated with three and a half litres of 7 per cent salt solution, stirred for three hours and

filtered through a battery of gravity filters. Toluene was used as an anti-septic and about two litres of clear extract was obtained in a couple of hours. The extract was acidified with 20 c.c. of 10 per cent acetic acid and placed in a boiling water-bath. The total globulins gradually precipitated and settled down. The precipitated globulins were filtered and washed by grinding with warm water. Finally, they were dehydrated by washing with alcohol and ether.

Fractionation of the globulins—Preliminary experiments showed that the saline extract contained two globulins which coagulated at two different temperatures, 43° to 45° C and 82° to 85° C. The extract after acidifying with acetic acid as before was gradually warmed in a water-bath. At 40° C there was a slight turbidity and coagulation commenced at about 43° C. The temperature of the water-bath was maintained at 43° C to 45° C for about one hour and the precipitated protein was filtered, washed and dehydrated with alcohol and ether as before. This was called globulin A.

The filtrate from the above was further warmed. When the temperature reached about 70° C there was a slight coagulation. This was filtered off and the filtrate heated further. Between 82° C to 85° C there was copious coagulation. The precipitate was allowed to settle, filtered, washed and dehydrated with alcohol and ether. This precipitate was called globulin B. The solution left after separation of these two fractions gave on boiling only a slight turbidity but no precipitate.

The quantity of globulin A was about one-fourth of globulin B. In each case two preparations were made of the above four proteins—prolamin, total globulin, globulin A and globulin B. The preparations were all powdered and passed through a 100-mesh sieve, and analysed. The elementary analyses of the preparations are as follows—

TABLE III

	Prolamin		Total globulin		Globulin A		Globulin B	
	Per cent.		Per cent.		Per cent.		Per cent.	
	1	2	1	2	1	2	1	2
Moisture	6.77	6.55	7.10	6.21	4.17	4.36	5.87	6.13
Ash	0.52	0.62	0.81	0.88	0.99	0.91	1.43	1.16
On ash and moisture free basis								
Nitrogen	15.16	15.10	15.34	15.18	14.96	14.99	16.21	16.21
Sulphur	0.62	0.64	0.80	0.84	1.15	1.09	0.68	0.79

The nitrogen distribution and essential amino-acid contents of the preparations are given in Tables IV and V. The nitrogen distribution was carried out by the method of Van Slyke as modified by Plimmer and Rosedale (1925). Cystine and arginine as estimated by the Van Slyke method are incorrect as has been shown by Plimmer and his co-workers (1925 and 1927). Therefore, arginine was estimated directly in the hydrolysate after removal of the amide nitrogen (Plimmer, 1916). Cystine, tyrosine and tryptophane were all estimated according to the method of Fohn and Marenzie (1929). In the case of cystine the modification of Remington (1930) was employed.

TABLE IV
Nitrogen distribution
(Expressed as per cent of total nitrogen)

Form of Nitrogen	Prolamin		Total globulins		Globulin A		Globulin B	
	1	2	1	2	1	2	1	2
Melanin—insoluble	2.07	1.98	0.99	0.97	2.17	1.83	1.25	0.98
Melanin—adsorbed by lime	0.81	0.92	1.02	1.06	0.86	1.09	0.86	0.92
Amide	20.97	21.02	7.08	6.96	6.29	6.29	7.03	7.14
<i>Basic</i>								
Arginine	3.33	3.52	10.03	10.17	10.45	10.66	19.06	19.02
Histidine	2.89	2.68	4.25	4.37	7.24	6.84	1.75	1.98
Cystine	0.75	0.72	1.12	0.93	1.31	1.45	0.74	0.60
Lysine	2.46	2.56	8.06	8.32	7.08	7.88	8.05	8.51
<i>Non basic</i>								
Amino	64.37	64.87	58.48	57.35	59.54	60.47	56.31	56.01
Non amino	1.71	2.29	4.01	3.63	6.17	5.13	4.91	4.02
Total	99.36	100.56	101.04	99.76	101.11	101.02	101.56	99.18

TABLE V

*Essential amino acids**(Expressed as per cent of ash and moisture-free protein)*

	Prolamin	Total globulins	Globulin A	Globulin B
Arginine	2.38	8.78	6.50	11.14
Histidine	1.55	2.44	3.89	1.12
Lysine	6.08	6.54	5.84	7.10
Cystine	2.24	3.02	3.80	2.50
Tyrosine	2.89	3.80	3.68	3.90
Tryptophane	2.07	0.75	0.95	0.70

The prolamin of *Bajra* is compared with the prolamins of other cereals in Tables VI and VII

TABLE VI

*Nitrogen distribution of prolamins from various cereals**(Expressed as per cent of total nitrogen)*

Form of nitrogen	Prolamins from						
	<i>Pennisetum typhoides</i>	<i>Eleusine¹ coracina</i>	<i>Trigonella² foenum graecum</i>	<i>Triticum³ vulgare</i>	<i>Zea³ mays</i>	<i>Andropogon³ sorghum</i>	<i>Sorghum³ vulgare</i>
Melamin	2.89	1.48	1.30	0.87	1.11	1.35	3.08
Amide	20.99	22.00	20.20	24.61	18.06	20.76	18.90
<i>Basic</i>							
Arginine	3.43	2.95	3.10	6.38	3.92	3.92	4.83
Histidine	2.79	2.30	0.70	5.41	2.45	1.71	1.19
Cystine	0.74	0.44	1.40	1.68	0.98	1.23	0.92
Lysine	2.51	0.59	0.70	0.57	0.89	2.48	3.45
<i>Non basic</i>							
Amino	64.62	68.92	70.20	53.49	66.08	68.85	60.01
Non amino	2.00	2.24	2.30	6.14	5.90	0.32	5.33

¹ Niyogi, Narayana and Dewai, 1934² Srinivasaro, Sastri and Narayana, 1933.³ Hoffman and Gortner, 1925

TABLE VII
Essential amino acids in various prolamins
(Expressed as per cent of protein)

Amino acids	<i>Pennisetum typhoideum</i>	<i>Trigonella¹ foenum graecum</i>	<i>Eleusine² coracina</i>	<i>Triticum³ vulgare</i>	<i>Zea³ mize</i>
Arginine	2.38	2.30	2.50		
Histidine	1.55	0.40	1.40		
Lysine	1.08	0.50	0.50		
Cystine	2.24	3.00	2.50	2.10	1.03
Tyrosine	2.80	4.30	5.30	3.30	5.88
Tryptophane	2.07	2.40	1.60	0.81	0.20

¹ Srinivasarao, Sastri and Narayana, 1933

² Niyogi, Narayana and Desai, 1931

³ Folin and Marenzie, 1929

The main differences between globulin A and globulin B are the higher sulphur, cystine and histidine contents of the former. Globulin B contains nearly double the arginine content of globulin A. Globulin B constitutes the major part of the total globulins and as such its analysis more nearly corresponds to that of the total globulins.

The prolamins of *Bajri* compares very favourably with those of the other cereals. It has a higher lysine content and resembles the prolamins of sorghum. Like other prolamins the *Bajri* prolamins are on the whole poor in the basic amino acids but are rich in tryptophane and cystine. The pulse globulins are usually poor in these amino acids. Therefore *Bajri* should form a good complement to the other pulses.

The cystine contents of all the proteins very nearly correspond to their sulphur contents.

Summary

Bajri contains about two-fifths of its proteins as a prolamins and one-fifth as a globulin. The latter can be fractionated into two proteins, a small part A coagulating at 43°-45° C and the larger part B coagulating at 83°-85° C.

The nitrogen distribution of prolamins and total globulin and its two fractions, globulin A and globulin B, have been studied and essential amino acids determined. Globulin A is richer in cystine and histidine but is poorer in lysine and arginine, the latter being half that in globulin B.

The prolamins compares favourably with the prolamins of other cereals. Being rich in tryptophane and cystine it should possess a good nutritive value and be a useful complementary protein to the pulse proteins which are poor in these amino-acids.

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STUDIES ON PHYSICO-CHEMICAL RELATIONS OF SOIL AND WATER

I. Water Retentive Force of Soil as influenced by Chemical Fertilisers

BY B N SINGH AND M L MEHTA

(From the Institute of Agricultural Research, Benares Hindu University)

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Introduction

SOIL moisture is a prime factor in successful plant growth. In previous communications^{1,2} from this Experiment Station published in this very periodical, the dominating influence of a proper supply and availability of water to crops has been emphasized. After having gained an insight into the relations of soil moisture to plant growth, attention has been further directed to studying some of the most fundamental physico-chemical relations of the water and soil, an aspect of considerable importance in the study of water relations of plants.

The normal functioning of a plant depends primarily on the constancy and the abundance of water supply through it. The enormous loss of water sustained by the plants in the process of transpiration necessitates a correspondingly energetic absorption of water from the soil. Consequently the proper development and health of plants depend primarily upon the capacity of the soil to deliver water to the plants. But as the moisture content decreases, the soil particles develop a 'force' or 'back pull' by virtue of which they tend to retain their water. The measurement of this force gives an estimate of the water retaining capacity of soils.

Since the time Cameron³ emphasized the importance of soil forces, the general status of our knowledge of the subject has been appreciably raised by the investigations of Shull and Livingston and his coworkers^{3,4}.

Shull³ from his investigations on the subsoil of Oswego silt loam concludes that as the soil becomes drier, the forces retaining water become greater, until at the approach of air-dry conditions a very small change in moisture content results in a huge change in the forces involved. Livingston and his co-workers^{3,4} worked with 12 types and mixtures of soils and concluded that as the soil mass dries out, its water supplying power decreases.

and these changes occur most rapidly in the immediate vicinity of plant roots and near the soil surfaces. The investigations of Shull and Livingston show that the capacity of the soil to deliver water to roots is one of the most important factors influencing the intake of water by plants. It is imperative that for successful crop production those conditions which ordinarily tend to reduce the water retentive force of the soil to the minimum, need be given. The main aim of the present investigation is to find out the effect of the common practice of adding artificial fertilisers to soil, on the water-retaining force of soils at different moisture percentage, i.e., how the different fertilisers affect the water-retaining force as the soil dries out.

Experimentation

For the determination of the water retentive power of the soil the osmotic solution method developed by Shull^{*} has been followed with minor modifications. The method consists in determining the amount of water that air-dry seeds with semipermeable coats extract, from the soils and from salt solutions of varying molar strengths. The osmotic pressure of the salt solution in which the water intake by seeds corresponds to the water intake from the soil represents the water retentive force of the soil.

The seeds used in the experiment came from a pure line stock of Sutton's Peas "Earliest of all" which were tested and found to have semipermeable coats.

The fertilisers were added in the following proportions: Ammonium sulphate (N) 2 per cent, * potassium sulphate (K) 1 per cent and double super phosphate (P) 0.5 per cent. The following moisture percentages besides the air-dry (2.5 per cent) soil, were maintained by adding that much volume of water to 100 gm. of air-dry soil: 25 per cent, 20 per cent, 15 per cent, 10 per cent, 5 per cent and the wilting coefficient. The wilting coefficient of each of the soils was found out by experimentally ascertaining the hygroscopic coefficient and dividing it by 0.68¹. These agreed fairly with the values found out by direct methods for all fertiliser effects and other field experiments going on at this Experiment Station.

100 gm. of soil of each of the types mentioned above were placed in 500 cc. screw-topped bottles having a rubber band at the neck for tight fitting. After keeping 100 sterilised and weighed seeds of as uniform a size as possible in the soil the bottles were tightly stoppered and kept agitated.

* Weight of fertiliser per 100 gm. of soil. This proportion of fertilisers has been found to be more or less optimum for the growth of crops from separate investigations at this place.

in a water-bath maintained at a temperature of $25^{\circ} \pm 1.0^{\circ} \text{C}$. After a series of trials four days time was found to be sufficient for the attainment of moisture equilibrium between seeds and soils. Longer durations of time introduced fungal infections in higher moisture contents. At the end of the experiment the seeds were carefully removed out of the soil, any adhering soil brushed out, the moisture content of both seeds and soils determined and the intake of water by the seeds calculated. Another series of air-dry seeds were placed in NaCl solutions of varying molar strengths and the percentage moisture they imbibed under these conditions determined. Two days time was found sufficient for the attainment of moisture equilibrium in salt solutions.

The intake of water in the different salt solutions is portrayed in Fig. 1 and that in different soils detailed in Table I. For the values of water intake

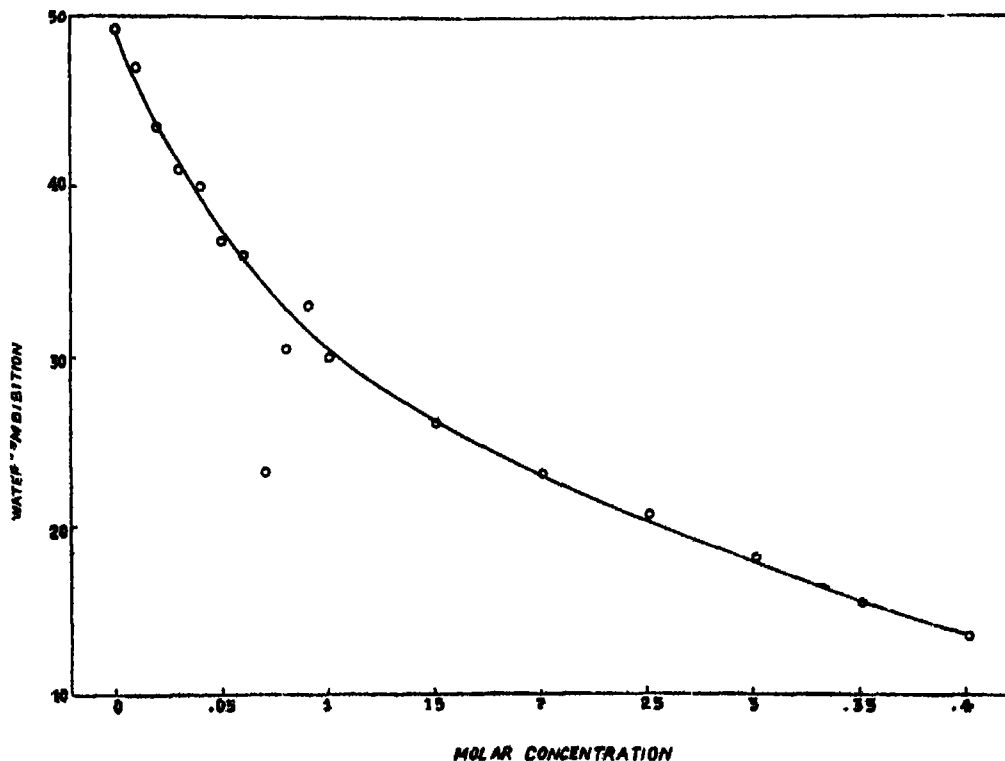


FIG. 1.—Imbibition of water by seeds from the various concentrations of sodium chloride solution

in different soils the corresponding strength of salt solution is looked from the graph (Fig. 1) and from the latter the pressure in atmosphere ascertained

TABLE I

Intake of water by Pisum seeds at various moisture percentages

No.	Treat- ment	Water absorbed at moisture percentage					
		2.5	5	10	15	20	25
1	Control	13.5	14.9	24.9	34.4	36.0	38.1
2	K	22.33	16.6	26.2	34.1	35.0	35.3
3	P		13.7	22.9	28.1	29.9	34.4
4	N		15.1	25.0	32.4	31.0	32.0
5	KP	13.2	15.6	24.3	29.6	32.1	34.2
6	PN	15.8	18.6	22.2	25.6	26.1	26.8
7	KN	17.5	18.9	27.1	28.5	28.7	29.2
8	KPN	20.0	22.1	23.8	24.3	25.4	25.6

from physical tables. These values are expressed as water retentive forces of soils.

Experimental Findings

Fig. 2 depicts the effect of decreasing moisture content on the water retentive force of soils. Considering the control it is noted that as the soil gradually dries from 25 to 2.5 per cent moisture, the water retentive force becomes greater and greater. At higher moisture contents the changes brought about in the force are steady but below 10 per cent moisture, large changes in the forces are involved. All the treatments show more or less similar relationships between the moisture content and water retentive force. At the higher moisture ranges like 25 and 15 per cent, K has practically no effect, differing as it is, very little from the control. At the level of the wilting coefficient and below, K exerts the greatest influence bringing the water retentive force to a value higher than the control itself. Among the other single treatments P brings about steady changes and keeps the force at the wilting coefficient, at the lowest level. In the double combinations only PN shows a steady change in this force, the enhancing effect of K on the water retentive force being again apparent in combinations KN and KP. The triple

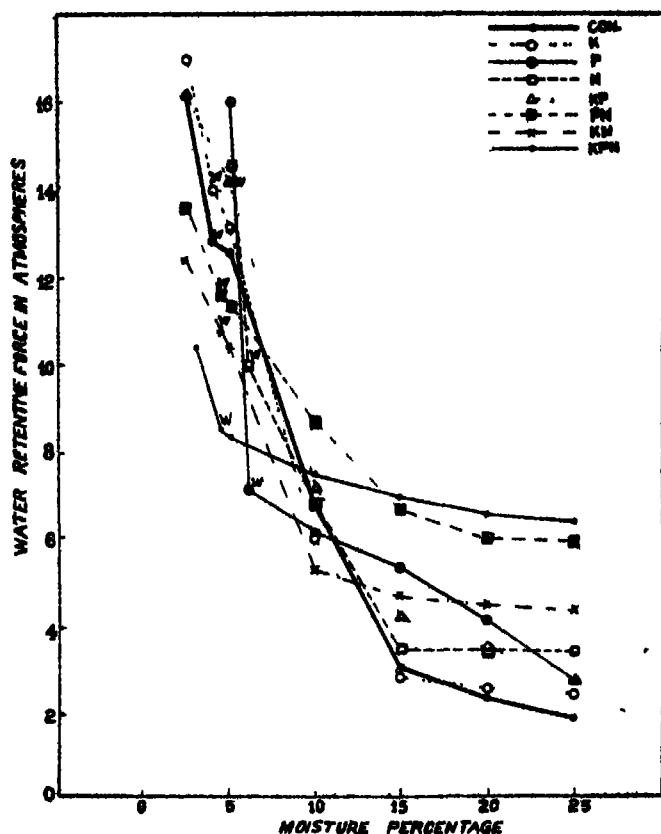


FIG 2 —Water retentive force of variously fertilised soils at different moisture contents
Points marked W represent the wilting coefficient

combination KPN shows the most level course, the effect of K in increasing the water retentive force presumably being checked by the opposite effects of P and N. A survey of the curves thus demonstrates that as the soil dries the water-retaining power of the soils also increases a result agreeing with the findings of Shull³ and Livingston^{3,4}. Double superphosphate (P) appears to have the best effect in checking the increasing water retaining forces with decreasing moisture contents and potassium sulphate (K) the least effect. Under the conditions of excessive moisture, K has no influence in checking the supply of water from soils to plants which would naturally result in a wastage of water by plants and in conditions of insufficiency of moisture the K treatment excessively increases the retentive force making it difficult for the plant to get a proper supply of water.

TABLE II
Increase in gradient of water retentive force from 25 per cent moisture to wilting coefficient

Treatment	Retentive force at wilting coefficient (dyne/cm)	Retentive force at 25% moisture	Gradient of increase in the force
Control	12.1	1.7	10.4
K	13.8	2.2	11.6
P	6.8	2.5	4.3
N	9.5	3.1	6.4
KP	14.0	2.5	12.5
PN	11.0	5.6	5.4
KN	10.2	4.1	6.1
KP	7.9	6.0	1.1

Fig. 3 and Table III give the relationship between the wilting coefficient and water retentive force in variously fertilised soils. It is observed that

TABLE III
Water-retaining force at wilting coefficient of the fertilised soils

Treatment	Hygroscopic coefficient	Wilting coefficient	Water-retaining force at wilting coefficient
Control	2.68	3.95	12.1
K	2.61	3.84	12.3
P	4.11	6.05	6.8
N	4.08	6.00	9.5
KP	3.25	4.88	12.3
PN	3.02	4.49	11.0
KN	2.98	4.39	10.2
KPN	3.11	4.50	7.9

both are greatly influenced by the addition of fertilisers. With increasing values of wilting coefficient from 3.85 (in case of K) to 6.2 (in case of P) there is a decrease in water retentive force from 12.5 to 6.8 atmospheres with the exception of KP, where although the wilting coefficient is higher

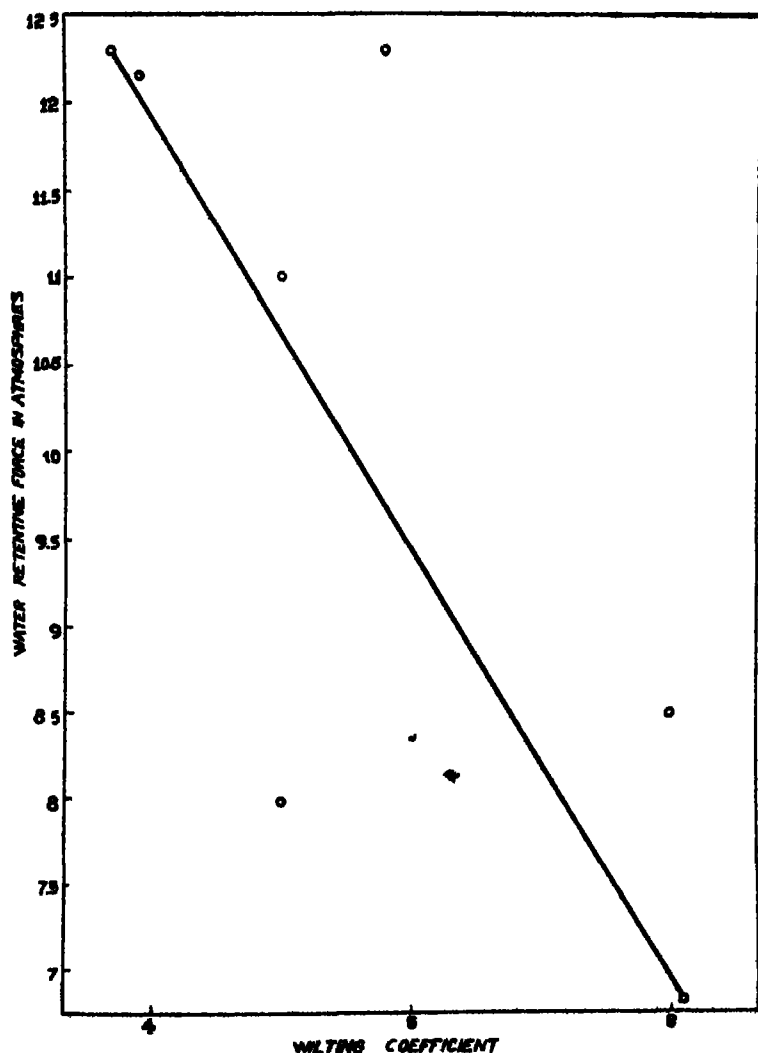


Fig. 3—Water retentive force of variously fertilised soils as related to the wilting coefficient

than KN, PN and KPN the force is not decreased but is higher than those. K thus tends to decrease the wilting coefficient and increase the water retentive force of the soil while P tends to increase the wilting coefficient and

decrease the water retentive force N has a balancing effect of these two opposing influences as evidenced by the intermediate value of KPN

Table II represents the gradient of increase in water retentive force from 25 per cent moisture to the wilting coefficient level in different treatments. The gradient is highest in the case of K treatment and least in KPN. The effectiveness of P is again evident as seen from the low gradient of increase in P and PN fertilised soils.

It is well known that for the proper intake of water by plants the root cells have the capacity to adjust their osmotic relations in accordance with the opposing forces in the soil. This capacity of roots has certainly a limitation and too high a force in the soil would demand a corresponding high change in the osmotic relations of the plant cell which often the plant may not be able to cope with. This would result in an upsetting of the water relation of the plant and its metabolism ultimately. From the data presented in Table II it is evident that the changes in the force brought about in the soil by the depletion of water to the stage of wilting coefficient is very high in K treated soils. Hence, in these soils, the plant has to make considerable changes in the osmotic relations of the root cells when the moisture content is reduced to low values. The small changes in the osmotic relations of the roots of plants that will be required in the N and P treated soils naturally emphasise their usefulness all the more.

The results presented in this paper on the effect of artificial fertilisers as an important factor influencing the water relations of plants, may be said to be in conformity with other independent investigations on their effect on the water requirements and yield of crops under Indian conditions. To mention but a few, in an yield trial experiment on potatoes⁶ the treatments KP and PN and N yielded 9.46, 9.63 and 8.79 tons per acre while K resulted in an yield (6.03 tons) less than that of the control itself (6.07), and the treatment PN in an investigation on wheat⁷ decreased the water requirement and increased the yield over the KP and KN treatments indicating thereby the effectiveness of PN additions to the soil on the yield and water requirement.

Summary and Conclusions

The paper deals with effects of ammonium sulphate (N), potassium sulphate (K) and double superphosphate (P), singly and in combinations (total 8 treatments) on the water retentive force of the soil at different moisture contents.

As the moisture content of the soil decreases the water retentive force of the soil increases. The changes in this force are less at higher moisture percentages while large changes are noted at lower moisture contents.

K has the least effect in reducing the water retentive force as soil is being depleted of its moisture, and P both singly and in combinations has the best effect in as far as it tends to keep the forces steady

The fertilisers influence both the wilting coefficient and water retentive force of soils (Table III) In general the fertilisers bringing about increase in wilting coefficient also bring about a corresponding decrease in water retentive force

The gradient of increase in water retentive force of soils from high to low moisture content is higher in K treated soils and low in P treated soils (P, PN and KPN) This necessarily calls for drastic changes in the osmotic relations of the root cells of plants in K treated soils if the plants have to maintain a proper water balance

It is concluded that a combination of P and N is the best treatment as far as water relations of plants are concerned for the proper growth and yield of crops under Indian conditions

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THE MYXOPHYCEÆ OF THE BIHAR PROVINCE, INDIA—I*

BY C SURYAPRAKASA RAO, B.Sc.

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[Communicated by Prof Y. Bhāradwāja, M.Sc., Ph.D (Lond.), F.L.S.]

ON the suggestion of Professor Y. Bhāradwāja, the author made some collection of Myxophyceæ in March 1938 from some of the chief towns in Bihar, namely, Patna,¹ Darbhanga,² Muzaffarpur,³ Samastipur⁴ and Madhubani⁵

In this communication, in all twenty-five forms have been recorded and out of these three varieties and three forms are new

* From the Department of Botany Benares Hindu University

¹ Patna is situated in latitude 25° 37' N and longitude 85° 10' E., at a height of 193 feet above sea-level. The hottest months, namely, April, May and June, have a mean maximum temperature of 98.1° F., the highest temperature record being 114° F. The coldest months are December, January and February and they record a mean minimum temperature of 52.3° F., the extreme minimum temperature being 36° F. June, July, August and September are the chief rainy months and during these months the rainfall amounts to 41.94", the annual rainfall being 48.53"

² Darbhanga is situated in latitude 26° 10' N and longitude 85° 54' E., at a height of 165 feet above sea-level. The hottest months, namely, April, May and June, have a mean maximum temperature of 94.6° F., the highest temperature recorded being 110° F. The coldest months are December, January and February and they record a mean minimum temperature of 52° F., the extreme minimum temperature being 34.2° F. June, July, August and September are the chief rainy months and during this period the rainfall amounts to 42.59", the annual rainfall being 50.27".

³ Muzaffarpur is situated in latitude 26° 07' N and longitude 85° 24' E., at a height of 177 feet above sea-level. The hottest months are April, May and June and they record a mean maximum temperature of 95.7° F., the highest temperature recorded being 109° F. The coldest months are December, January and February and they record a mean minimum temperature of 50.3° F., the extreme minimum temperature recorded being 36.2° F. June, July, August and September are the chief rainy months and the rainfall during these months amounts to 41.58", the annual rainfall being 48.85".

⁴ Samastipur.—The chief rainy months are June, July, August and September and during this period the rainfall amounts to 42.45", the annual rainfall being 49.34".

⁵ Madhubani.—June, July, August and September are the chief rainy months, the total rainfall during this period being 43.21". The annual rainfall is 51.07".

SYSTEMATIC ENUMERATION OF THE SPECIES OBSERVED

I CHROOCOCCALES

Chroococcaceæ

Genus *Microcystis* Kuetzing

1 *Microcystis flos-aquæ* (Witttr) Kirchn Smith, 'Ecology of the Plankton Algæ of the Palisades Interstate Park,' *Roosevelt Wild Life Bulletin*, Vol 2, No 2, 1924, Pl 3, Fig 13, Gettler, in *Rabenhorst's Kryptogamenflora von Europa*, Band XIV, Cyanophyceæ, 1930-32, p 136, Fig 59 e and f

Lat cell, 3-5 5 μ (-6 μ)

Habitat —In a tank, Patna

Genus *Aphanothece* Naegeli

2 *Aphanothece pallida* (Kutz.) Rabenh Gettler, *op cit*, 1930-32, p 172, Fig 78, Frémy, "Les Myxophyceæ de l'Afrique équatoriale française," *Archives de Botanique*, III (1929), Mem 2, 1930, p 33, Fig 31

Lat cell, 5 5 7 μ , long cell, 6 6 10 μ

Habitat —In a pond, Darbhanga

Genus *Merismopedia* Meyen

3 *Merismopedia glauca* (Ehrenb) Nag Smith, "The Plankton Algæ of the Okoboji region," *Transactions of the American Microscopical Society*, Vol XLV, No 3, 1926, Pl 1, Fig 5, Gettler, *op cit*, 1930-32, p 264, Fig 129 d, Frémy, *op cit*, 1930, p 14, Fig 9

Forma (Fig 1, A)

Lat col, 75-85 μ , long col, 100-110 μ , lat cell, 4 5-5 5 μ

Habitat —In a pond along with *Azolla*, *Oedogonium* sp, *Spirogyra* sp
Darbhanga

The form differs from the type in having eighty as the most common number of the cells included in a colony, but occasionally there may be even bigger colonies consisting of upto 320 cells formed by the apposition of four smaller colonies

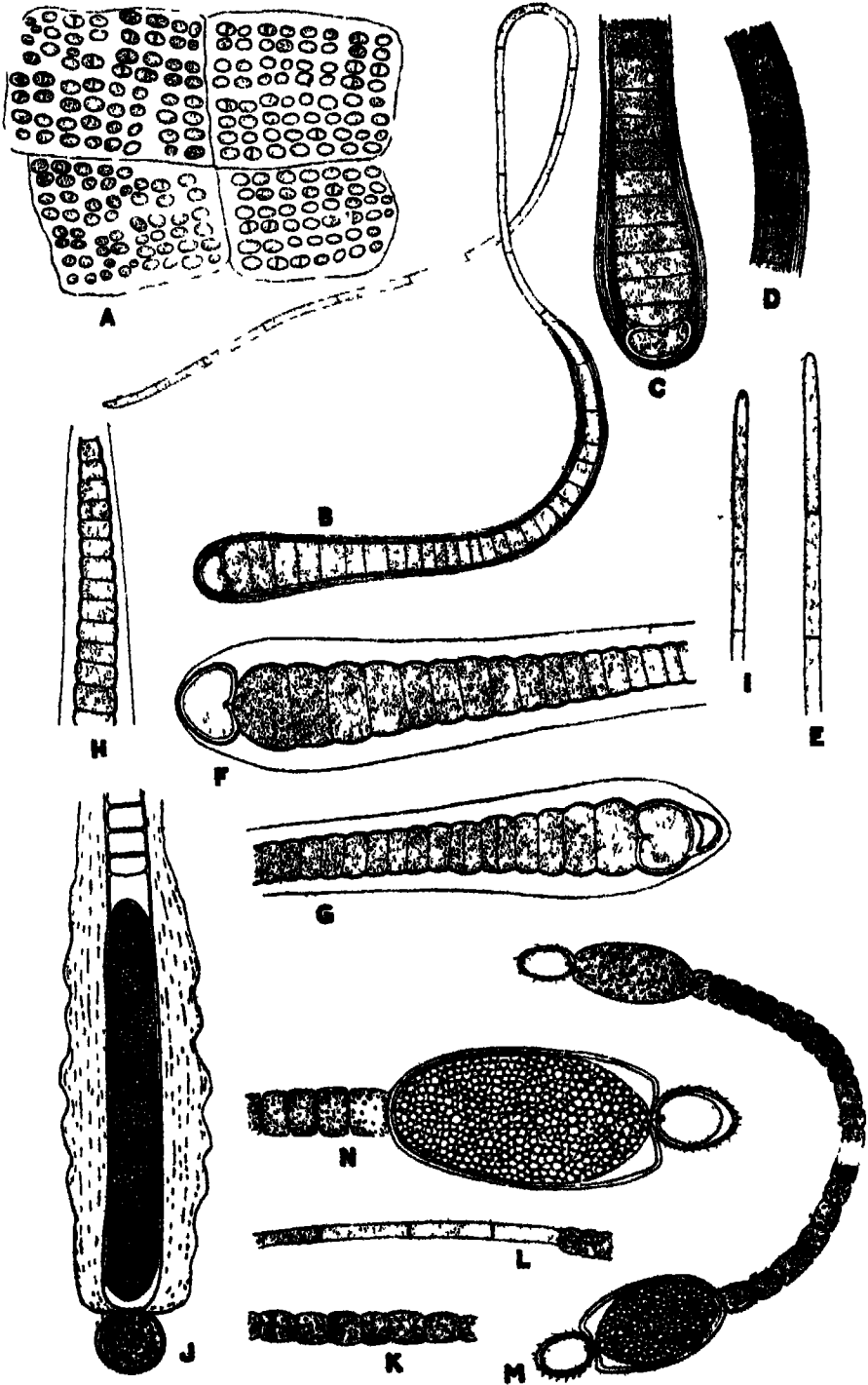


FIG. 1

1.—*Merismopedia glauca* (Ehrenb.) Näg. forma, a big colony formed by apposition of four small colonies, B—entire plant, C—base, D—middle and E—terminal portions of the filament of *Calothrix fusca* Born et Flah var *crassa* var nov, F—Normal, G—with a second crushed heterocyst, H—middle and I—terminal portions of *Calothrix Castellii* (Massal.) Born et Flah var *samastipurensis* var nov, J—basal, K—middle and L—cells of the hair of *Calothrix Raichowshi* Woloszynska var *longispora* var nov, M—entire plant, N—single spore magnified of *Cylindrospermum indentatum* (G. S. West) forma *major* form nov B and M $\times 600$ the rest $\times 1,080$

II HORMOGONIIDS

(a) Rivulariaceae

Genus *Calothrix* Agardh

- 4 *Calothrix fusca* Born et Flah Frémy, *op cit*, 1930, p 246, Fig 222
var *crassa* var nov (Fig 1, B—E)

Filaments single, irregularly bent or straight Sheath yellowish-brown, stratified, closely adpressed to the trichome Trichomes without constrictions at the septa and tapering into a long hair, the terminal portion of which is generally unsheathed Heterocysts basal, single and hemispherical No spores

Long trich, upto 900μ , lat fil, $16-18\mu$ lat trich, at base $10-12\mu$, higher up $4-6\mu$ and at apex 2μ , long cell, 3.6μ , lat het, 10μ , long het, $6-8\mu$

Habitat —Occurring along with *Aphanothece pallida* in a pond, Darbhanga

The variety agrees with the type in occurring singly along with other algae, in possessing unconstricted trichomes with bulbous base and tapering into a long hair, and in basal hemispherical heterocysts enclosed by the sheath, but it differs from the type in having broader trichomes, much longer hair and yellowish-brown sheath*

- 5 *Calothrix Castellii* (Massal.) Born et Flah Geitler, *op cit*, 1930-32, p 611, Fig 385, Frémy, *op cit*, 1930, p 255, Fig 227

var *samastipurensis* var nov (Fig 1, I—J)

Thallus calcareous and cushion-like Filaments closely interwoven Sheath colourless, unstratified Trichomes swollen at the base, slightly constricted at the septa and tapering into a long hair Heterocysts basal single, occasionally in pairs, hemispherical, with slightly drawn-out apices No spores

* Since the dimensions of the heterocysts in the type are not recorded, it is not possible to compare them with those of the variety

Long fil, upto 1,100 μ , lat fil, 10-20 μ , lat trich, at the base upto 14 μ , higher up 6-6 μ , at the apex 2 μ long cell, at the base 3-8 μ , higher up 4-6 μ and in the hair 4-12 μ , lat het 6-12 μ long cell, 4-10 μ , crass vag, upto 4 μ

Habitat —On the cemented floor under a water-tap on the railway platform, Samastipur

The variety agrees with the type in all respects except that the former has shorter filaments, broader trichomes, longer cells and heterocysts with slightly drawn-out apices, which may occasionally occur in pairs. Moreover, the Samastipur form is a cold water alga

Genus *Gleotrichia* Agardh

6 *Gleotrichia Raciborskii* Woloszyńska Gentler *op cit*, 1930-32, p 637, Fig 405 a and b

var *longispora* var nov (Fig 1, J-L)

Thallus irregularly lobed, 2-5 cm thick. Trichomes with constrictions at the joints, ending in long hair. Cells at the base barrel-shaped or quadratic, as long as broad or shorter, higher up, cylindrical or barrel-shaped, in the hair long cylindrical, heterocysts single, spherical or ellipsoidal. Spores long cylindrical, with a smooth outer wall

Lat trich, at the base 7-4-9-5 μ , higher up 4-6 μ , at the apex 2 μ , long cell, at the base 8-12 μ , higher up 4-10 μ , in the hair 14-16 μ , lat het, 12-14 (-16) μ , lat spor cum vag, 22-28 μ , lat spor, 10-12 μ long spor, 80-122 μ (average 100 μ), crass vag, 6-9-2 μ

Habitat — Planktonic in a pond, Darbhanga

The variety agrees with the type in all respects except that the former has broader trichomes, bigger heterocysts, narrower and much longer spores and indistinctly stratified sheath. It can also be compared with var *kashiense* Rao (C Bhashyakarla Rao, "The Myxophyceae of the United Provinces, India -III" *Proc Ind Acad Sci*, Vol VI, Sec B, No 6, 1937, p 351, Fig 3 A-E) on account of the cell dimensions and the nature of the heterocysts, but, it differs from the same in having bigger cells at the base and much longer spores. A comparison can also be made with var *conica* Dixit (Dixit, "The Myxophyceae of the Bombay Presidency, India -I," *Proc Ind Acad Sci* Vol III, Sec B, No 1, 1936, p 97, Fig 1, G and H), but the form differs from it in having bigger heterocysts and longer spores

(b) Nostocaceæ

Genus *Cylindrospermum* Kuetzing

7 *Cylindrospermum indentatum* G. S. West (Gentler, *op cit*, 1930 32, p 820, Fig 523 a

Forma *major* form nov (Fig 1, M and N)

Lat trich, 5.5–6 μ , long cell, 5.6–12 μ , lat het, 7–10 μ , long het, 11–16 μ , lat spor, 16–18 μ , long spor, 30–40 μ

Habitat —Floating in a canal along with *Zanichellia* and *Ceratophyllum* leaves, Muzaffarpur

The form differs from the type in having broader trichomes, bigger heterocysts and bigger spores. The trichomes possess heterocysts and spores at both ends.⁷

Genus *Nostoc* Vaucher

8 *Nostoc spongiforme* Ag. Frémy, *op cit*, 1930, Fig 279, a and b, (Gentler, *op cit*, 1930 32, p 839, Fig 531

Lat cell, 3.5–4 μ , long cell, 4–10 μ , lat het, 4–6 μ , long het 6–10 μ , lat spor, 6–8 μ , long spor, 8–10 μ

Habitat —Attached to moist rocks in the river Gandack, Muzaffarpur

Genus *Anabaena* Borv

9 *Anabaena spherica* Born et Flah. var *attenuata* Bhāradwāja Bhāradwāja, "The Myxophyceæ of the United Provinces, India—I," *Proc Ind Acad Sci*, Vol II, Sec B, No 1, 1935, Fig 5, G and H

Lat trich, 3.3–5 μ , long cell, 3.3–6 μ , lat het, 4.8–6.4 (8) μ , lat spor, 14–16 μ ,

Habitat —In a stagnant pond, Muzaffarpur

⁷ The occurrence of heterocysts and spores at both ends of trichomes has by now been reported in many species of the genus *Cylindrospermum*, namely, *C. licheniforme* Kütz, *C. maius* Kütz, *C. minutissimum* Collins, *C. eulanatum* Italf, *C. Gelzin* Schmidt, var. *binum* Borge, *C. muscicola* Kütz var *variabilis* Borge and var *kashmirensis* Bhāradwāja and *C. indica* Rao. On the basis of this character Bhāradwāja (cf Bhāradwāja "Contributions to our knowledge of the Myxophyceæ of India" *Annals of Botany*, 1933, 47, 177) has suggested the creation of a separate section *Cylindrospermopsis* of the genus *Cylindrospermum*. If this were to be followed the present form may have to be referred to some other species belonging to this section and not to *C. indentatum*, since West does not mention the occurrence of spores and heterocysts at both ends of the trichome in the Egyptian alga.

Besides having a single spore on either side of a heterocyst, this form may sometimes possess a pair of spores on either side of a heterocyst, or one spore on one side and two on the other

10 *Anabaena Iyengar* Bhâradwâja, Bhâradwâja, *op cit*, 1935, p 106, Fig 6, H-K

Forma

Lat cell, 3.5-4 μ , long cell, 3-4 μ , lat het, 4 μ , long het, 4.5-5.4 μ lat spor, 4.7-8 μ , long spor, 9.1-14.8 μ

Habitat Free-floating in a pond along with other algæ, Darbhanga

The form differs from the type in having much smaller heterocysts and narrower spores. It agrees with var *tenuis* Rao in the size of the cells and the spores, but differs in having intertwined trichomes, smaller heterocysts and chains of spores instead of a single spore on either side of a heterocyst

(c) *Oscillatoraceae*

Genus *Spirulina* Turpin

11 *Spirulina platensis* (Nordst.) Gom var *tenuis* Rao C Bhashyakarla Rao, *op cit*, 1937, p 365, Fig 7, A and B

Lat trich, 4-6 μ , lat spir, 30-43 μ , spat inter duo spir, 40-55 μ

Habitat —Free-floating in a tank along with the other algæ, Darbhanga

Genus *Oscillatoria* Vauch

12 *Oscillatoria angusta* (Bory) Gom Gertler, *op cit*, 1930-32, p 945, Fig 599, Tilden, *Minnesota Algæ*, Vol 1, 1910, Pl IV, Fig 9

Lat trich, 6.7-2 μ , long cell, 2.5-4 μ

Habitat —In a waste water drain, Patna

13 *Oscillatoria tenuis* Ag Gertler, *op cit*, 1930-32, p 960, Fig 611 g, Tilden, *op cit*, p 71, Pl IV, Figs 17 and 18, Pr  my, *op cit*, 1930, p 217, Fig 186

Lat cell, 8-10 μ , long cell, 2.2-5 μ

Habitat - On moist soil along with *O formosa*, Darbhanga

14 *Oscillatoria terebriformis* Ag Gertler, *op cit*, 1930-32, p 955, Fig 607 d

Lat trich, 3.3-4 μ , long cell, 3-5 μ

Habitat.—On moist ground, Patna.

15 *Oscillatoria formosa* Bory Gertler, *op cit*, 1930-32, p 970,
Fig 619 b, Tilden, *op cit*, 1910, p 80, Pl IV, Fig 33

Lat trich, 4-6 μ , long cell, 2-4 μ

Habitat —On moist soil along with *O tenuis*, Darbhanga

16 *Oscillatoria Borvana* Bory Gertler, *op cit*, 1932, p 954,
Fig 607 c

Lat trich, 6-8 μ , long cell, 3-6 μ

Habitat —On wet soil, Patna

17 *Oscillatoria brevis* (Kutz) Gom Gertler, *op cit*, 1930-32, p 946,
Tilden, *op cit*, 1910, p 79, Pl IV, Fig 32, Frémy, *op cit*, 1930, p 225,
Fig 195

Lat trich, 4-6 μ , long cell, 2.5-3.5 (-4 μ)

Habitat —On moist edges of a tank, Madhubani

18 *Oscillatoria sancta* (Kutz) Gom Frémy, *op cit*, 1930, p 210,
Fig 177, Tilden, *op cit*, 1910, Pl IV, Fig 5, Gertler, *op cit*, 1930-32,
Fig 598 c

Lat trich, 9.9-12 μ , long cell, 2-3.5 μ

Habitat —Free-floating in a pond along with other algæ, Madhubani

19 *Oscillatoria claricentrosa* Gardner Gertler, *op cit*, 1930-32, p 964,
Fig 615 c

Lat cell, 2-2.5 μ , long cell, 4.5-6 μ

Habitat —Occurring along with sterile *Zygnema* filaments and *O tenuis*,
Madhubani

20 *Oscillatoria pseudogeminata* G Schmidle Gertler, *op cit*, 1930-32,
p 966, Fig 616

Lat cell, 1.6-2 μ , long cell, 3-5 μ

Habitat —On moist soil, Madhubani

Genus *Phormidium* Kutz

21 *Phormidium Retzi* (Ag) Gom Gertler, *op cit*, 1930-32, p 1012,
Fig. 647, a-d

Lat fil, 5-6 μ , long cell, 3.3-5 μ

Habitat —On moist edges of a drain, Patna

22 *Phormidium dimorphum* Lemm Gertler, *op cit*, 1930-32, p 1012, Fig 639

Lat cell, 5-6 μ , long cell, 2-4 μ

Habitat —On moist soil in a drain, Patna

23 *Phormidium incrustatum* (Nag) Gom Gertler, *op cit*, 1930-32, p 1017, Fig 649a

Lat fil, 4-5 μ , lat trich, 2.5-3 μ , long cell, 2.8-5.6 μ

Habitat —Attached to a decaying log of wood in a tank, Samastipur

Genus *Lyngbya* Agardh

24 *Lyngbya majuscula* Harvey, var *chakrænce* Rao C Bhashyakarla Rao, "The Myxophyceæ of the United Provinces, India—II," *Proc Ind Acad Sci*, Vol III, Sec B, 1936, p 174, Fig 3, E and F

Lat fil, 40-50 μ , lat cell, 35-40 μ , long cell, 3.5-7.5 μ , crass vag, 2.5-6.5 (10) μ

Habitat —Free-floating in a fresh-water tank, Darbhanga

Genus *Microcoleus* Desmazieres

25 *Microcoleus cthonoplastes* Thuret Gertler, *op cit*, 1930-1932, p 1134, Fig 739

Diam vag, 40-100 μ , lat vag, 15-35 μ , lat cell, 3.3-4.5 μ , long cell, 4-8 (-10) μ

Habitat —On moist soil under a tree adjoining a pond, Muzaffarpur

In conclusion, I have much pleasure in expressing my great indebtedness to Professor Y Bhāradwāja, for his kind guidance and criticism throughout the course of this investigation

THE INFLUENCE OF ARTIFICIAL FERTILISERS UPON THE PHOTOSYNTHETIC EFFICIENCY OF *ANDROPOGON SORGHUM*

BY B N SINGH, K N LAL AND M B LAL

(From the Institute of Agricultural Research, Benares Hindu University)

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Introduction

THE relative importance of nutritive ingredients specially nitrogen, phosphoric acid and potash in regulating plant growth has been studied by a number of investigators (1, 4, 10, 12, 16, 18, 21, 22, 23 and 31) Ample evidences have been put forth to show that the different fertilizing constituents induce characteristic variations in the carbohydrate metabolism of plants (3, 7, 8, 13 and 24) The pigment content of leaf tissues has also been found to vary considerably in relation to the presence or absence of these elements (28, 32, 33, 34, 35)

Direct evidences on the influence of fertilizers upon the photosynthetic appropriation of CO_2 by the leaves, however, are still lacking And although the fundamental importance of different mineral ingredients in carbon assimilation has been well stressed by a number of investigators (2, 6, 9, 14, 15 and 17) not much is known regarding the concentration and combination under which these will have maximum augmentative effect

The investigations reported in the following pages were mainly conducted to throw light in these directions and serve to elucidate in its various aspects, the influence of nitrogen, phosphoric acid and potash when supplied in form of different fertilizers upon the photosynthetic efficiency of leaves

Methods and Material

The experiments were conducted on *Andropogon sorghum* (Var Cawnpore 46) Glazed culture pots 18" $\times$ 12" in size were filled with well-sterilized clay-free sand. Seeds of uniform size and weight were selected and sown at the rate of ten per pot The three fertilizing ingredients, namely, nitrogen, phosphoric acid and potash, were then added in the form of neutral ammonium sulphate (20% nitrogen), double superphosphate (40% P_2O_5), and potash sulphate (48% K_2O). The rate of application of these fertilizers per pot in different series was as follows :

Series I Control—Containing no nitrogen, phosphate or potash.

Series II Single fertilizer cultures.

A *Nitrogen series*—

- (i) Containing 1 0 gm ammonium sulphate per pot
- (ii) Containing 3 0 gm ammonium sulphate per pot
- (iii) Containing 6 0 gm ammonium sulphate per pot
- (iv) Containing 9 0 gm ammonium sulphate per pot

B *Phosphoric acid series*—

- (i) Containing 0.4 gm superphosphate per pot
- (ii) Containing 2 0 gm superphosphate per pot
- (iii) Containing 3 6 gm superphosphate per pot

C *Potash series*—

- (i) Containing 0 4 gm potassium sulphate per pot.
- (ii) Containing 2 0 gm potassium sulphate per pot
- (iii) Containing 2 8 gm potassium sulphate per pot
- (iv) Containing 3 6 gm potassium sulphate per pot

Series III Three fertilizers cultures

- (i) 'Full-manure' pots, each containing 6 0 gm of ammonium sulphate, 2.0 gm of superphosphate and 2 0 gm of potassium sulphate
- (ii) 'Double-manure' pots each containing 12.0 gm of ammonium sulphate, 4 0 gm of superphosphate, 4.0 gm of potassium sulphate
- (iii) 'Half-manure' pots each containing 3 0 gm ammonium sulphate, 1.0 gm superphosphate, 1 0 gm potassium sulphate
- (iv) 'One-fourth manure' pots each containing 1 5 gm ammonium sulphate, 0 5 gm superphosphate, 0.5 gm potassium sulphate

Series IV Deficiency fertilizer cultures *

A *Nitrogen deficiency series*—

- (i) 1/10 N_2 deficient
- (ii) 1/20 N_2 deficient
- (iii) No nitrogen

* Optimal level of N_2 , K_2O and P_2O_5 is identified with 6 gm ammonium sulphate, 2 gm. potassium sulphate, 2 gm. superphosphate (double) The level of deficiency is calculated on this basis. All other fertilizers except the one under deficiency was maintained at optimal level.

B Phosphoric acid deficiency series—

- (i) 1/10 P_2O_5 deficient
- (ii) 1/20 P_2O_5 deficient
- (iii) No phosphate

C Potash deficient series—

- (i) 1/10 K_2O deficient
- (ii) 1/20 K_2O deficient
- (iii) No potash

Fertilizers were supplied in one doze previous to sowing

Iron and manganese in the form of ferric chloride and manganese sulphate were supplied in traces equally in all the series of cultures

Equal amount of water was added to each of the pots and moisture content maintained in the vicinity of 35 to 38% water-holding capacity (30) This saturation limit in the soil was kept constant throughout the period of experimentation by the addition of an amount of water equivalent to that lost during transpiration or evaporation from the pot

When the seedlings were established thinning was done and only four healthy plants of equal vigour were allowed to grow per pot The pots were numbered and arranged in regular rows in the pot house

When the seedlings were a month old their rate of assimilation and chlorophyll content were determined Photosynthesis was measured by the continuous current method (29) and the values of assimilation expressed in milligrams of CO_2 per 100 sq cm area or per unit fresh weight of the leaf tissue The chlorophyll content was determined according to Oltman's method (25) and expressed in milligrams per 10 gm fresh weight of the material

The rate of photosynthesis was determined under optimal conditions of light, temperature, and CO_2 supply so as to reduce the limitations due to factors other than the fertilizing ingredients

Experimental Findings

A. Single Fertilizer Series Experiments

The data obtained on the photosynthetic rate of seedlings in different series of cultures (Table I) indicate in general that increase in the application of fertilizers upto a certain definite concentration results in an increase in photosynthetic activity Thus in case of 'single-fertilizer N_2 series' photosynthesis gradually increases from 4 551 mgm in the control to 8 131 mgm (Expts I and IV), when as much as 6 gm of the nitrogenous fertilizer has been added to the culture. Application of quantity higher than this, viz.,

9 gm per pot, has a definite deleterious effect so much so that the rate of CO_2 influx is reduced to 4 628 mgm (Expt V)

In case of 'single-fertilizer K_2O series' even small quantities of potash sulphate is found to increase the photosynthetic rate much above the value recorded for nitrogen application. In this case photosynthetic rate continuously rises to an optimal limit in cultures supplied with 2 gm potash sulphate (Expt VII). Higher quantities of this fertilizer of the order of 2.8 to 3.6 gm per pot even, bring about a sudden fall in photosynthesis. The relative value of such application, however, as compared to the control is always higher even in cultures supplied with as high as 3.6 gm per pot potash sulphate (Expt XII).

In contrast to potash, phosphates increase the photosynthetic efficiency of leaves upto a wider range so much so that even 3.6 gm per pot of super-phosphate does not bring down the photosynthetic rate at all.

Considering the relative value of the three different fertilizers from the point of view of photosynthetic augmentation it is evident that when supplied singly, potassic and phosphatic fertilizers are much more useful than the nitrogenous. This is indicated by the fact that under optimal condition of application the percentage increase over the control is 78.66% in nitrogen, 575.43% in potash and 465.49% in phosphate.

B. Three Fertilizer Series Experiments

From what has been noted previously it may safely be concluded that for inducing optimal photosynthetic activity applications of 2.0 gm of ammonium sulphate, 2.0 gm potash sulphate and 2.0-3.6 gm of super-phosphate is absolutely necessary. In the next series of experiments, therefore, a mixture of these fertilizers was supplied.

A perusal of Fig 1 indicates that in plants grown under the so-called optimal supply of fertilizers, photosynthesis does not vary to any appreciable extent as compared to that recorded for the control. In 'half-manure' series there is a decided increase in photosynthesis by 172.27% over the control. Further reduction in manuring to one-fourth the optimal limit (Expt XVI), however, reduces the photosynthesis below the value attained in 'half-manure' plants, but decidedly above that of the 'full-manure' ones.

In none of these cases does the rate of photosynthesis reach the same high intensity as recorded under optimal doses of potash or phosphates in the 'single-fertilizer' series. This indicates that the three manures when present in the same substratum do not allow photosynthetic augmentation to the same extent as noted in the case of optimal doses of phosphate or potash applied singly.

C Deficiency Series Experiments

In all the 'deficiency series' elements other than those maintained deficient were supplied in optimal doses such that photosynthetic variations may be mainly attributed to the mineral ingredient kept in deficiency

In the ' N_2 -deficient' series the rate of photosynthesis is found to slightly increase in response to partial deficiency of nitrogen (Expts XVII and XVIII) Further increase in nitrogen deficiency upto $-1/20$ the optimal value has a deleterious effect inasmuch as photosynthesis is brought back to same level as that observed for the control (Expts I and XVIII) It is curious to note however, that in completely nitrogen-starved plant photosynthesis is maximum in the whole group This in itself indicates that application of nitrogenous fertilizers somehow brings down the photosynthetic efficiency of the leaf

In the potash deficient series, deficiency of this mineral ingredient is found to be definitely useful in initiating a higher rate of photosynthesis (Expts XXIII and XXIV) In $-1/20$ potash deficient plants photosynthesis increases by approximately 2.19 and 2.20 times over the respective values of fully manured and control plants Completely potash-starved plants assimilate much more beyond the level observed for $-1/20$ K plants, being 259.7% more efficient than the fully manured ones

Under phosphorus deficiency also photosynthesis varies in more or less the same direction as observed in the case of potassium deficient plants The results obtained in deficiency series thus indicate in general that the association of the three elements in concentration found optimal (cited above) is always deleterious from the point of view of photosynthesis When one or the other element is either reduced or completely removed this deleterious influence is partly overcome and photosynthesis increases

Fertilizer Effect on Respiration and Apparent Assimilation of Leaves

To throw further light on the question at tissue the rate of real assimilation of leaves under the different manurial treatments have been compared with the apparent assimilation and respiration values under these very conditions.

The rate of apparent assimilation varies in much the same way as the rate of real assimilation observed under different manurial treatments But variations are found to occur in the concentration under which optimal effect is observed (Table I and Fig. 1) The optimal effect in single fertilizer series is noted when these fertilizers (nitrogenous, potassic and phosphatic) are supplied to the extent of 3.0 gm. ammonium sulphate, 0.4 gm. potash

TABLE I

Variations in Photosynthetic Rate (Apparent and Real), Respiration, and Chlorophyll Content of Andropogon sorghum in Response to Different Fertilizer Treatments

Temperature = $31^{\circ}\text{C} \pm 0.2$

CO_2 concentration = 0.30 to 0.35 per 100 c.c.

Illumination, 1000 half watt Philips bulb kept at 18 cm distance

Single fertilizer series

Expt No	Treatments	CO ₂ in mgm per 100 sq cm leaf area per hour			Chlorophyll in mgm per 10.0 gm fresh weight
		Apparent assimilation	Respiration	Real assimilation	
I	Control with no treatment	1.542	3.009	4.551	3.85
<i>Single fertilizer N₂ series</i>					
II	1.0 gm Ammonium sulphate	1.479	3.946	5.425	18.51
III	3.0 gms	3.710	2.267	5.977	17.44
IV	6.0	2.439	5.692	8.131	19.05
V	9.0	1.157	3.471	4.628	10.71
<i>Single fertilizer P₂O₅ series</i>					
VI	0.4 gm double superphosphate	10.279	5.870	16.149	14.70
VII	2.0 gm	10.465	9.156	19.621	15.07
VIII	3.6	19.198	6.434	25.532	16.28
<i>Single fertilizer K₂O series</i>					
IX	0.4 gm Potassium sulphate	22.213	2.099	24.312	7.40
X	2.0 gm	20.274	10.464	30.739	8.69
XI	2.8	2.935	5.751	8.686	4.84
XII	3.6	2.484	3.864	6.348	4.17
<i>Three fertilizer series</i>					
XIII	'Full manure'	2.706	1.870	4.576	4.46
XIV	'Double manure'	2.201	8.071	10.272	20.63
XV	'Half manure'	6.688	5.731	12.419	25.64
XVI	'One-fourth manure'	2.507	3.912	6.419	18.96

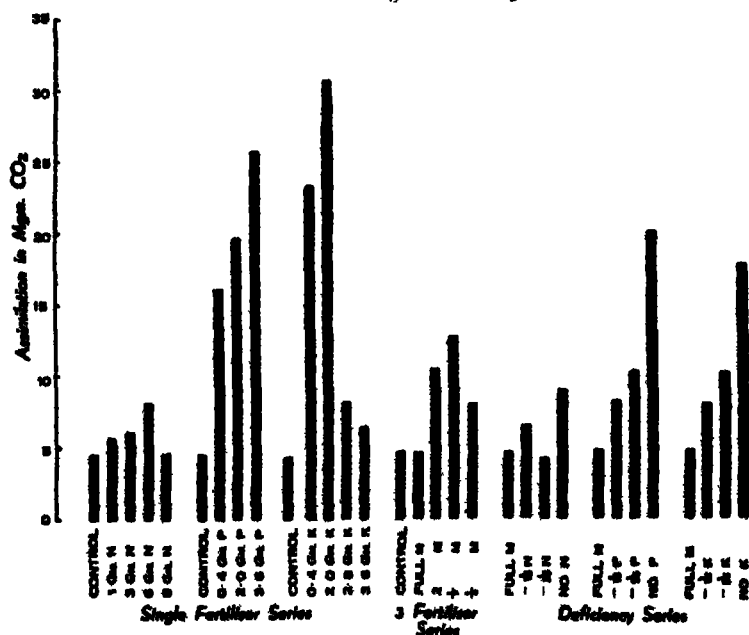
TABLE I (Contd.)

Deficiency series*

Expt No	Treatments	CO ₂ in mgm per 100 sq cm leaf area per hour			Chlorophyll in mgm per 10.0 gm fresh weight
		Apparent assimilation	Respiration	Real assimilation	
<i>N₂ deficiency series</i>					
XVII	1/10 N ₂ deficient series	4.888	1.505	6.393	15.15
XVIII	1/20 „ „ „	0.098	3.499	4.197	5.00
XIX	No nitrogen	4.387	4.388	8.775	15.02
<i>P₂O₅ deficiency series</i>					
XX	1/10 P ₂ O ₅ deficiency series	1.157	6.943	8.100	20.00
XXI	1/20 „ „ „	2.200	7.800	10.000	21.00
XXII	No phosphate	11.222	8.596	19.818	25.03
<i>K₂O deficiency series</i>					
XXIII	1/10 K ₂ O deficiency series	5.800	1.744	7.632	16.66
XXIV	1/20 „ „ „	4.458	5.572	10.030	20.55
XXV	No potash	6.500	11.000	17.500	23.55

* For details of the quantities of fertilizers added in each series refer to pages 152 and 153

N.B.—The data in these columns are the average of three separate estimations



sulphate and 3.6 double superphosphate respectively as against 6.0 gm., 2.0 gm and 3.6 gm in case of real assimilation

In 'full-manure' plants the rate of apparent assimilation is only 25.57% (Expt XIII) of the value recorded for control Under 'double

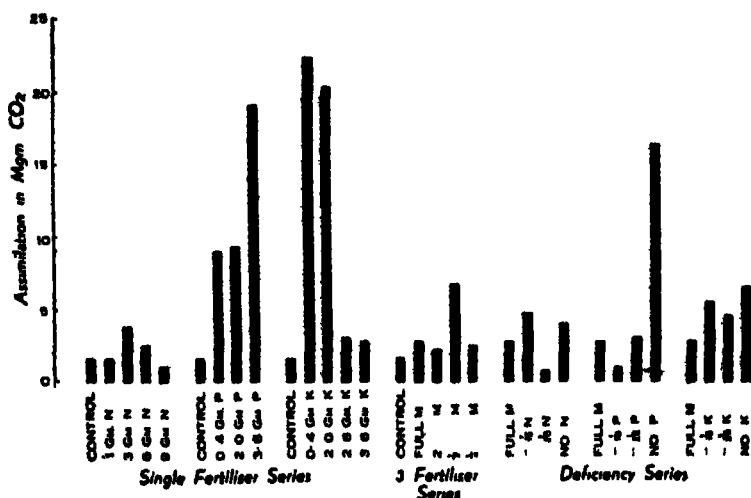


FIG 2

Fertiliser effect on the apparent assimilation rate of young leaves

manure' (Expt XIV) apparent assimilation is still further reduced. Reduction of fertilizers to half the quantity supplied under 'full-manure' series, however, increases the intake of CO₂ (Expt XV). Reduction to one-fourth the full manure level again brings down the apparent photosynthetic rate to a level much lower than that obtained in full manurial plants.

In deficient series, deficiency of nitrogen and potash up to 1/10 the full manured level increases the rate of apparent assimilation beyond the values recorded for the full manure plants. Under complete deficiency (Expt XIX) apparent assimilation again increases much beyond the value recorded for either the full manure or 1/20 deficient plants. In case of phosphorus, apparent photosynthesis slightly decreases up to 1/10 P₂O₅ deficiency but later follows the usual rise with increasing deficiency.

So far as respiration is concerned the optimal level of fertilizers under which maximum respiration takes place is located in cultures supplied singly with 6.0 gm of ammonium sulphate, 2.0 gm of potash sulphate and 3.0 gm of superphosphate (Fig 3). In the three fertilizer series increase of fertilizers to double the optimal level gives the best result, compared with this, reduction to half or one-fourth the full manure level reduces the rate of respiration.

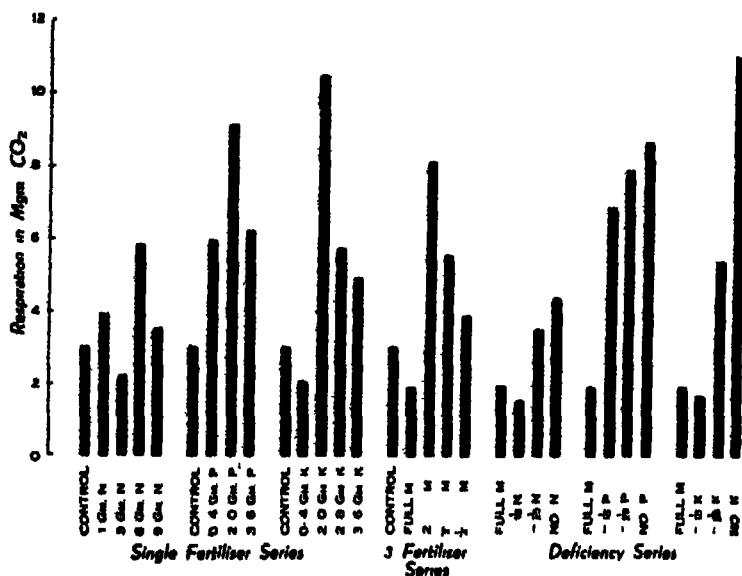


FIG 3

Fertiliser effect on the respiration rate of young leaves

In 'deficient' series respiration continuously rises with increase in the deficiency of different ingredients

The data thus indicate that the influence of fertilizers upon respiration and assimilation (both apparent and real) is practically of the same nature. The anomalies observed only in a few cases might well be attributed to factors other than the manurial ingredient themselves.

Fertilizers Effect on Chlorophyll Content

Wide variations in the chlorophyll content of leaves are observed in response to the different treatments. In the single fertilizer series application of nitrogenous fertilizers has the best effect upon the chlorophyll content of leaf, next in order being phosphatic and potassic fertilizers respectively (Table I and Fig 4).

With increase in the quantity of either of these fertilizers, chlorophyll content increases upto a certain optimal limit, this being located in cultures supplied with (i) 6.0 gm. of ammonium sulphate, (ii) 2.0 gm. of potassium sulphate, and (iii) 3.6 gm. of superphosphate respectively.

In the 'three-fertilizer series' plants receiving the fertilizer to half the optimal level exhibit the highest chlorophyll content. Further reduction to 'one-fourth' the optimal limit reduces the chlorophyll content of leaves below that obtained for 'half-manure' plants. But even in this case the

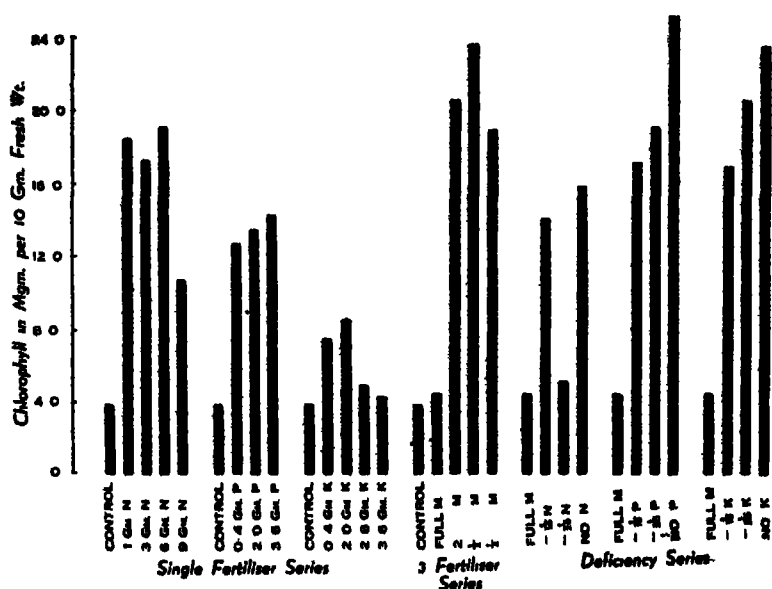


FIG. 4

Fertiliser effect on the chlorophyll content of young leaves

concentration of chlorophyll is much higher as compared to the control or the full manured plants

In 'deficient series' deficiency of nitrogen upto 1/10 the optimal level increases the chlorophyll content, whereas in $-1/20$ nitrogen deficiency series there is definite reduction in its value. Potash and phosphorus deficiency on the contrary, definitely increase the chlorophyll content even upto $-1/20$ of the optimal value. Completely starved plants belonging to either the nitrogen, phosphorus, or potash deficient series are much better than all others in this respect.

Comparing the entire series of experiments, it may incidentally be remarked that plants receiving 6.0 gm of ammonium sulphate and 2.0 gm. potassium sulphate only contain the maximum amount of chlorophyll in their leaves. Next in order are the 'half-manure' plants receiving 3.0 gm of ammonium sulphate and 1.0 gm each of potash sulphate and superphosphate.

Discussion

The foregoing observations lead to the generalisation that application of different fertilizers when supplied either singly or in different combinations greatly influences the photosynthetic efficiency, respiratory rate and chlorophyll content of leaves.

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Under the influence of certain fertilizer combinations, augmentation in assimilatory activity is also followed by increase in chlorophyll content or acceleration in respiratory activity. To cite a few instances, the highest photosynthetic activity in response to 2.0 gm potassium sulphate in the single fertilizer series is accompanied by an increase in chlorophyll content, and the respiration rate beyond the values recorded for either other doses of potash itself or the completely unmanured plants. Again the maximum chlorophyll content observed in 'half manure' plants in the three fertilizer series is associated with high respiratory activity and maximum photosynthetic rate in whole of the series. In the deficiency series the maximum chlorophyll content in completely phosphorus starved plants is again associated with a maximum assimilatory activity and respiratory rate (Expt XXII). These experiments tend to indicate that the different fertilizers have specific effect upon the rate of respiration (6, 19, 20, 26) and the photosynthetic efficiency of leaves.

To analyse the specific nature of physiological response to the application of these fertilizers in all the different series of experiments, the rates of assimilation have been plotted against the concentration of different fertilizers (Fig 5)

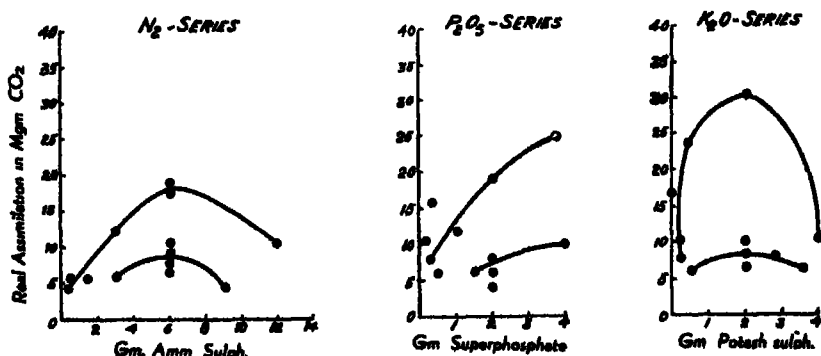


FIG. 5

The rate of real assimilation in young leaves under the influence of different fertilizers

It is of interest to note that majority of points arrange themselves in a regular order so as to yield characteristic curves in each case. Under the influence of each of these fertilizers two separate yet regular curves are obtained. The general trend of the curves indicate that in response to either nitrogenous, potassic or phosphatic manures assimilation increases at least upto a certain optimal level this being located under, (i) 6.0 gm of ammonium sulphate, (ii) 2.0 gm of potash, and (iii) 3.6 gm of superphosphate later showing a decline with increase in the quantity of fertilizer supplied.

When the log values of photosynthesis are plotted against the log value of different fertilizers (Fig 6) the curves appear to be distinctly of the parabolic

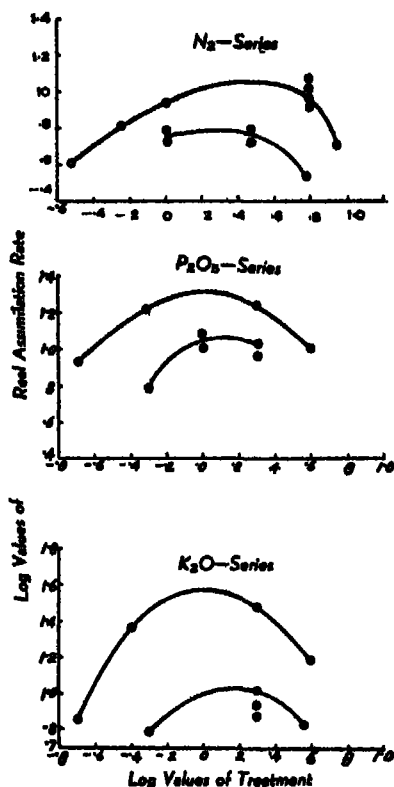


FIG 6

Log photosynthesis plotted against log values of the different fertilisers applied

type. In this case also two separate curves under the influence of each fertilizer are obtained similar to that indicated in Fig 5. The arrangement of points in two separate curves reveals the fact that under the influence of different doses of one single fertilizer may it be nitrogenous, potassic or phosphatic, the response is not always the same. Higher or lower rates of assimilation are induced depending upon the concentration in which factors other than the one under study are available. Thus, keeping the dose of phosphatic and potassic fertilizer constant, a variation in the nitrogen dose from 1/10th to 1/20th the optimal level, as under the nitrogen deficiency series, varies the photosynthetic rate from 6.393 to 4.197 mgm. (Table I, Expts XVII and XVIII). Similarly when the dose of phosphatic or potassic fertilizer is varied singly, the other two ingredients remaining constant, as

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in Expts XX-XXI and Expts XXIII-XXIV respectively, variations in intake of CO_2 are noted. It appears, therefore, that it is not the concentration or dose of one factor and one alone that is responsible for a particular rate of photosynthesis but that under similar doses of the same ingredient, variations in photosynthetic rate might take place depending upon the extent to which other complimentary factors are available. As to how the relative availability of different nutritive ingredients influences the photosynthetic process, shall be discussed at length in a subsequent contribution.

The relation between the chlorophyll content and the assimilatory activity of leaves has also been studied. When the chlorophyll value is plotted against the photosynthetic rate in various series of cultures, the curve tends to exhibit a level phase upto a concentration of 2.0 gm of chlorophyll per unit fresh weight of leaves (Fig 7). Beyond this concentration, however,

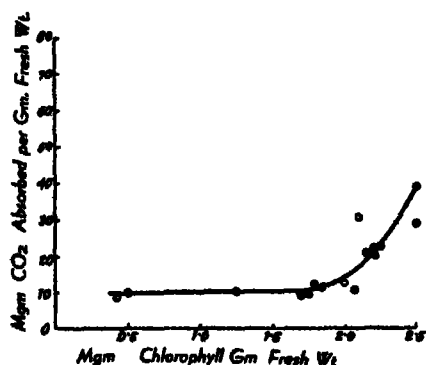


FIG 7

Photosynthetic appropriation of carbondioxide against the chlorophyll content of leaves

an increase in chlorophyll content is associated with a rise in photosynthetic efficiency of leaves. This indicates that in cultures inducing accumulation of chlorophyll beyond 2.0 mgm/fresh weight only photosynthetic rate is proportional to the concentration of chlorophyll (*cf* Emerson 5). In the flat region, factors other than the chlorophyll content, might have set a limit to the photosynthetic rate.

The assimilation number obtained after the method of Willstatter exhibit wide fluctuations (Table II). This indicates that in all the series of experiments, the chlorophyll content of the leaf tissue is not the only factor controlling photosynthesis. No direct proportionality between the presence of chlorophyll and photosynthetic activity is noted. The variations in assimilation number from series to series may partly be attributed to the

TABLE II

Variations in Assimilation Number of Andropogon sorghum in Response to Different Treatments

I Treatments*	II Mgm of CO ₂ per gm fresh weight	III Mgm of chlorophyll per gm fresh weight	IV Assimilation number II/III
Control	10.315	0.38	26.79
1.0 gm N	12.033	1.85	6.50
3.0 gm N	10.481	1.74	6.01
9.0 gm N	9.627	1.07	8.98
0.4 gm P	25.713	1.47	17.48
2.0 gm P	13.572	1.56	8.72
3.6 gm P	61.801	1.62	37.83
0.4 gm K	56.558	0.86	65.04
2.0 gm K	55.274	0.86	63.40
2.8 gm K	14.724	0.48	30.42
3.6 gm K	9.478	0.41	22.71
6.0 N, 2.0 K, 2.0 P gm	16.469	0.44	36.94
12.0 N, 4.0 K, 4.0 P gm	30.073	2.08	14.43
3.0 N, 1.0 K, 1.0 P gm	24.830	2.56	9.68
1.5 N, 0.5 K, 0.5 P gm	12.034	1.88	6.37
0.6 N, 2.0 K, 2.0 P gm	12.786	1.51	8.43
0.3 N, 2.0 K, 2.0 P gm	10.274	0.50	20.27
0.0 N, 2.0 K, 2.0 P gm	15.316	1.56	9.60
6.0 N, 2.0 K, 0.2 P	11.700	2.00	5.85
6.0 N, 2.0 K, 0.1 P	21.000	2.20	9.53
6.0 N, 2.0 K, 0.0 P	39.017	2.50	15.58
6.0 N, 0.2 K, 2.0 P	14.043	1.66	8.43
6.0 N, 0.1 K, 2.0 P	22.564	2.05	10.97
6.0 N, 0.0 K, 2.0 P	23.500	2.20	10.68

* For details refer to pages 152 and 153.

NB—'N' stands for ammonium sulphate, 'K' for potassium sulphate and 'P' for double super phosphate and the values of these fertilizers are in gm

presence or absence of N₂, K₂O or P₂O₅ in different series of experiments, although it does not bear any direct relation either with the dose or the combinations in which the different fertilizers are supplied. In response to the application of different fertilizers, thus variations are introduced in both the chlorophyll content and the photosynthetic rate of leaves. This indicates that the fertilizers influence photosynthetic machinery in one or the other following ways (i) they induce chlorophyll formation to varying extent and thereby indirectly influence the synthesis of carbohydrates, (ii) they

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have some direct influence upon the photosynthetic process in which case they may well be said to supply the three essential elements nitrogen, phosphorus and potassium for the proper functioning of leaf material and (iii) they increase the respiratory activity of the tissues which indirectly influences the rate of real assimilation

Summary

The influence of fertilizers upon photosynthesis, chlorophyll content and respiration rate of *Andropogon sorghum* (Var Cawnpore 46) has been studied under different combinations and levels of manuring

When supplied singly potassic and phosphoric fertilizers are much more useful than the nitrogenous in increasing the photosynthetic efficiency of leaves. In all these cases photosynthesis rises with increasing supply of fertilizers upto a certain optimal level, later showing a decline

In the three fertilizer series the rate of photosynthesis never reaches the same level as that recorded for optimal doses of potash and phosphates applied singly. The association of the three ingredients N_2 , P_2O_5 and K_2O appears to slacken the rate of CO_2 intake by leaves. When one or the other nutritive ingredient is either reduced or completely removed, this deleterious influence is partly overcome and photosynthesis increases

The influence of fertilizers upon respiration, photosynthesis (both apparent and real) and chlorophyll is practically of the same nature, inasmuch as, augmentation in assimilatory activity in majority of cases is also followed by an increase in chlorophyll content and respiration rate

Photosynthesis in different series of cultures does not rise with the increase in the chlorophyll content upto a critical limit of 2.0 mgm/fresh weight. Beyond this level, however, increase in chlorophyll is always associated with acceleration in photosynthetic activity

The variation in assimilation number in different series of experiments indicates that chlorophyll is not the only factor controlling photosynthesis

Photosynthetic augmentation under the influence of fertilizers may be due to (i) increased chlorophyll formation which in its turn influences carbohydrate metabolism, (ii) direct supply of mineral ingredients, nitrogen, phosphorus and potassium for proper functioning of leaf material and (iii) the increase in respiratory activity of leaves which considerably influences the rate of real assimilation

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PHOTOSYNTHETIC EFFICIENCY OF LEAVES AS INFLUENCED BY VARIATIONS IN pH OF THE INJECTED SOLUTIONS

BY B N SINGH AND S R A N. RAO

(From the Institute of Agricultural Research, Benares Hindu University)

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Introduction

THE importance of hydrogen-ion concentration in determining the fertility of the soil and regulating plant growth has been realized by a number of investigators^{2,12}. Various plant processes, such as enzyme action, formation and stability of proteins, and uptake of nutrients are conditioned by the concentration of H-ions inside the tissues as well as in the external medium^{8,11}. Meyerhof,¹⁰ Gustafson,⁷ Brooks,⁴ Bode,¹ Thomas¹⁷ and Lemmon⁹ studied the relationship between hydrogen-ion concentration and respiration of different materials. Treboux,¹⁸ and Bose³ noted that very dilute solutions of acids exerted a stimulating effect on photosynthesis. Direct evidences, pertaining to the effects of phosphoric acid (H_3PO_4) solution in certain concentrations upon photosynthesis of leaves, were put forth elsewhere¹⁶.

With the object of studying, in greater detail, the effect of H-ion concentration upon the process of photosynthesis, leaves* of *Saccharum officinarum* (Var. Co 312) were injected with different buffer solutions of known composition as indicated below

(1) Sodium acetate-acetic acid buffer —

pH 4.0 = 164 c.c. N/5 acetic acid + 36 c.c. N/5 Na acetate

pH 5.5 = 59 c.c. N/5 acetic acid + 141 c.c. N/5 Na acetate

pH 6.5 = 9 c.c. N/5 acetic acid + 191 c.c. N/5 Na acetate

(2) Borax-boric acid buffer (5) —

pH 7.5 = 12 c.c. M/20 borax + 188 c.c. M/5 H_3BO_3 + NaCl.

pH 8.5 = 55 c.c. M/20 borax + 145 c.c. M/5 H_3BO_3 + NaCl

The assimilation rates of control and treated leaves were determined after the manner described elsewhere¹⁸. The rate of intake of CO_2 in the experimental leaves injected with solutions of varying pH, was compared against the photosynthetic rate of control leaves of similar age and developmental stage but injected with freshly boiled and cooled distilled water.

* 3rd leaf on the main (primary) tiller of about 60 days old plant.

The complications due to CO_2 diffusion as a result of injection thus were brought to more or less similar level in both experimental and control leaves. The variations in assimilation rate between the different sets are therefore attributed in the main to the variations in the pH of the solution. The pH of the external solution and of the leaf sap were noted at the end of the injection-period (of about 12 hours). The sap was extracted, for the determination of its pH, by pressure after pounding the leaf in a glass mortar. The pH values were measured electrometrically by means of Hartmann and Braun 'Pehavi' using calomel and antimony electrodes.

Results

The data have been entered in Tables I and II. At the outset, all the treated leaves are found to exhibit lower rates of photosynthesis than the control (15.21 gm of CO_2 , Expt 1) the variations ranging from 16.57% to 78.30% of the control (Table I and Fig 1). Both apparent assimilation and respiration rates are also depressed considerably below the respective controls, except under pH 8.5 where the respiration of the treated leaf is 125.50% of the control (Table I and Fig 1).

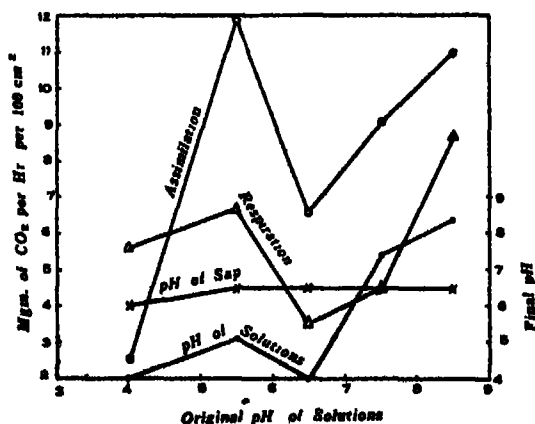


FIG 1

Relation between pH of the solutions, and (i) respirations and assimilation rates and (ii) the pH of the leaf sap and buffer solutions

For obtaining the acid range from pH 4.0 to pH 6.5 sodium acetate-acetic acid buffers have been used. Under pH 4.0 the leaf shows a photosynthetic rate of 2.52 mgm of CO_2 (Expt 2). Under pH 5.5 the rate increases to 11.91 mgm or 78.30% of the control (Expt 3 and Fig 1), which is the highest value reached in the treated series of leaves. Later on a decline in the value to 43.19% of the control is observed at pH 6.5 (Expt 4).

TABLE I

*Rates¹ of respiration and assimilation of control and treated leaves² of
Saccharaurn officinarum (Var Co 312)*

Light: 1250 C P 'Osram' bulb at 22 cm from the chamber

Temperature: $31.5 \pm 0.2^\circ \text{C}$ CO_2 . 0.38%

No	Treatment	Water content %	Respiration		Apprent assimilation		Real assimilation	
			Mgm of CO_2	% of control	Mgm of CO_2	% of control	Mgm of CO_2	% of control
1	Nil*	73.73	6.94	100.00	8.27	100.00	15.21	100.00
2	pH 4.0	73.86	5.58	80.40	-3.06	-37.00	2.52	16.57
3	pH 5.5	75.50	6.71	96.99	5.20	62.88	11.91	78.30
4	pH 6.5	73.14	3.46	49.86	3.11	37.60	6.57	43.19
5	pH 7.5	72.51	4.54	65.42	4.53	54.78	9.07	59.63
6	pH 8.5	68.44	8.71	125.50	2.26	27.33	10.97	72.12

*pH of cell sap = 6.0

Borax-boric acid buffers² were employed for obtaining pH 7.5 and pH 8.5. The photosynthetic rate at pH 7.5 is 9.07 mgm (Expt 5) and increases to 10.97 mgm or 72.12 % of the control (Expt 6) under pH 8.5.

The march of respiration also more or less closely follows the variations in photosynthesis under the different treatments (Table I and Fig 1). The rate is 5.38 mgm of CO_2 at pH 4.0 and increases to 6.71 mgm under pH 5.5, followed by a decline at pH 6.5 (Expts 2-4). There is, again, an ascending phase under pH 7.5 and 8.5, the values being 65.42 % and 125.50 % of the control respectively (Expts 5 and 6).

Similar variations are noted in the case of apparent assimilation rates also (Table II), with the exception of a decline in the value at pH 8.5.

The pH values of the expressed sap (Table II and Fig 1) of the control and of the leaf injected with pH 4.0 solution remain at 6.0. From pH 5.5 onwards upto pH 8.5 the sap remains constantly at pH 6.5. Apparently, therefore, there is no relationship between the variations in assimilation and respiration values and the pH of the cell-sap. But the final H-ion concentrations of the buffers (Table II and Fig 1) clearly exhibit a definite relationship.

¹ Rates of respiration and assimilation recorded are the mean values of three readings and expressed as the mgm. of CO_2 per hour per 100 sq. cm. of leaf surface.

² Leaf on the tiller of about 60 days old plant.

TABLE II

The pH of Buffers and leaf-sap before and after the period of Injection (of about 12 hours), and the extent of injury to the leaf

No	pH of buffers		pH of leaf sap		Extent of injury, as % of leaf area
	Initial	final	Initial	final	
1	4.0	4.0	6.0	6.0	54.50
2	5.5	5.1	6.0	6.5	33.60
3	6.5	4.0	6.0	6.5	9.28
4	7.5	7.4	6.0	6.5	0.00
5	8.5	8.4	6.0	6.5	52.00

with the changes in the influx and outgo of CO_2 in the leaves. A similar observation was recorded by Singh and Mitra¹⁴ regarding the growth of plants which was found to depend to a large extent upon the 'final' hydrogen-ion concentration of the medium. The buffer solutions of pH 4.0, 5.5, 7.5 and 8.5 show little or very slight shift at the end of the injection-period. In the case of pH 6.5 solution a great shift occurs towards acidity (i.e., to pH 4.0). Robbins¹⁵ found little change in pH of suspending buffers between pH 5.5 and 6.2. Similarly Lemmon⁶ observed very little reaction change in buffers with initial pH between 5.0 and 7.0.

Considering the sodium acetate-acetic acid buffer alone the concentrations of the solutions are very high (Fig. 2) and the consequent toxic effects upon photosynthesis are exhibited by the retarded rates already cited (Table I). Lemmon⁶ found such "Salt effects" upon the respiration of potato tissue, that is, some effects other than those caused by variations in pH, under quite concentrated buffer solutions. It was shown elsewhere¹⁶ that phosphoric acid (H_3PO_4) solution in certain concentrations have a stimulating effect on photosynthesis. On the basis of this and also the works of Treboux¹⁷ and Bose¹⁸ it is considered that acidity has a stimulating effect within a certain range, but, in the present instance, such an effect has been, to a great extent, antagonised by the high concentrations of the buffers. Probably these two antagonistic factors reach some sort of an "Equilibrium point"^{6,19} at pH 5.5, leading to slightly higher assimilation and respiration rates. At this stage the rates are still below the control values probably on account of the injurious effects of the solutes upon the leaf, as evidenced by the occurrence of a discoloured area to the extent of 33.6% of the total leaf-area (Table II). The injury appears to be caused by acetic acid. This

is further supported by the fact that the extent of injury lessens with the decrease in the amount of acetic acid in the solution (Table II and Fig 2)

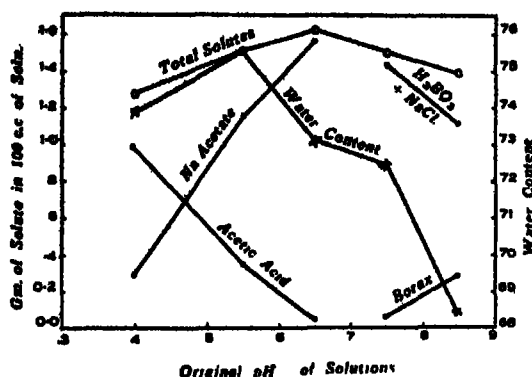


FIG 2

Relation between the original pH of the solutions, and (i) the number of grammes of solutes in buffers and (ii) water content of leaves

In the borax-boric acid buffers the concentration of the solutes seems to be toxic, but the photosynthetic rates have a tendency to remain higher than in the other treatments. With the increase of alkalinity from pH 7.5 to 8.5 there is a rise in photosynthesis from 9.07 mgm to 10.97 mgm of CO₂ (Expts 5 and 6). Respiration also increases with alkalinity of the buffers and at pH 8.5 the value surpasses (125.50%) of the control. Lemmon⁹ also noted a gradual stimulation in respiratory rate with the increasing alkalinity of the borax-boric-acid buffers from pH 7.5 to 9.5. At pH 7.5 the buffer solution appears to be quite 'balanced' with regard to its composition since no apparent injury to the leaf is noted (Expt 4, Table II). At pH 8.5, however, the leaf is definitely injured, the discolouration occurring to the extent of 52.00% of the total leaf-area (Expt 5, Table II).

Water content of the control and treated leaves (Table I) is within a known range of variations and does not appear to influence the photosynthetic or the respiratory rates to any significant extent.

Summary

The paper deals with the effects of injection of buffer solutions, varying in pH from 4.0 to 8.5, upon the photosynthesis and respiration of leaves of *Saccharum officinarum* (Var. CO 312).

A close relationship is found to exist between the photosynthetic and respiratory rates and the 'final' pH of buffers, although the reaction of the cell-sap is practically constant.

The high concentrations of the buffer solutions are, probably, responsible for depressing the rates below the values of the control, and for causing discoloured areas in the leaves in certain cases.

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THE REPRODUCTION, OOGENESIS AND DEVELOPMENT OF *MESOPODOPSIS ORIENTALIS* TATT.

BY K BHASKARAN NAIR, M Sc

Research Fellow, Department of Zoology, University of Madras

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Introduction

THE present work is an attempt to study the development of a tropical Mysid in the light of the new orientation given to the whole subject of Crustacean embryology by the important researches of Cannon, Sollaud and Manton during the last few years. The fact that very little is known about the reproduction and early development of the Crustacea and particularly the Mysidacea of tropical waters has led me in the course of this work to investigate these aspects of the life-history of *Mesopodopsis orientalis* in considerable

detail In tracing the later development and organogeny, the endoskeleton and musculature have been omitted and the observations on the other systems will be presented as briefly as possible, stressing those points where they differ from the findings of previous workers, though a continuous series of stages have been examined and carefully verified.

It gives me great pleasure to take this opportunity to thank Professor R. Gopala Aiyar, Director, University Zoological Research Laboratory, Madras, for his constant help and very valuable criticism I also desire to acknowledge here my indebtedness to the University of Madras for awarding me a Research Studentship to carry out this investigation

Historical Resumé

Though the earliest work on the embryology of the Mysidacea is that of van Beneden (1869) it is very seldom quoted by later workers except for the description of discoidal cleavage in *Mysis*, an observation which is even now considered exceptional Nusbaum (1887) working on the embryology of *Mysis chameleo* also upheld the view of van Beneden regarding segmentation and gave a fairly long account of the whole development He described and figured the changes in the external shape of the embryo and gave some details of organogeny Most of these latter have been shown to be inaccurate by Manton (1928) Bergh (1893) dealt with only the early development and was the first to describe the teloblastic formation of the post-naupliar ectoderm and mesoderm in *Mysis flexuosa* and *Mysis neglecta*. He failed to see the exact origin of the naupliar mesoderm and overlooked the presence of the preantennular mesoderm rudiments The work of Boutchinsky (1890) on *Parapodopsis cornuta* and of Wagner (1890) on *Neomysis vulgaris* have not been available to me Manton's (1928) work on *Hemimysis lamornei* is a very important contribution to Crustacean embryology She has traced the entire development from the four-celled stage up to the formation of the adult structures Besides correcting the numerous errors of previous workers and bringing light and order into a much confused field, the account of the origin and growth of a definite preantennular somite and the formation of the heart, the demonstration of segmental coelomic cavities and of an embryonic seventh abdominal segment, among other important things, stand as valuable contributions to Zoology from her None of the above authors has studied the reproductive processes or traced the history of the egg before it starts upon the developmental cycle

Bionomics

Mesopodopsis orientalis occurs in large shoals in the brackish-water adjoining the mouth of the river Adyar in Madras The sheet of water is

mostly shallow and not very extensive and is in free communication with the sea from October-November to February-March. During the rest of the year it is separated from the sea by a sand-bar. By about the middle of October the North-East Monsoon begins and the river floods the place with fresh water. The temperature at the river mouth ranges from 77° F to 85° 37° F and the salinity varies from 16 l gm to 30 44 gm per mille.* *Mesopodopsis orientalis* is known to brave much wider ranges of salinity. There is no doubt that it lives and breeds in the sea, for, the plankton of the Madras Coast commonly shows brooded females of the species. Tattersall (1915) says that it ascends some of the larger rivers on the east coast of the Indian Peninsula for a distance of at least 40 miles above tidal influence and that in many places it occurs in water that is permanently fresh. At Adyar it could be collected in great plenty most of the year, though in the monsoon months the shoals disappear and collections become very poor. For one or two months a random specimen or two are the only catch and there are weeks on record when no specimens were available. By the beginning of January shoals make their appearance, but it is a common experience that they, at this time, contain only very young specimens mostly of the same age. Adult shoals are not at all common at this time. This fact indicates a rhythm in the reproductive cycle of the species, but the data available are too insufficient to make any definite statement.

The adult Mysid is a transparent and delicate creature very active in its movements and measuring about 7.6 mm in length. Very often the specimens of Adyar show huge bushy colonies of Zoothamnid ciliates, attached all over the body, but especially crowded at the posterior end. Except for the bizarre appearance, the specimens bearing these seem to suffer from no inconvenience whatsoever. They are as healthy and move about as actively as the uninfested ones. The antennal gland is clearly seen in the living specimen when examined under the microscope. It is large and tubular, lying in the shape of a bent U at the anterior end of the body. In lateral view it is found to stretch from the base of the antenna to the mid-dorsal line. The end sac is situated between the mandible and the base of the antenna.

It has invariably been found that approximately 96 hours after the extrusion of the eggs the young ones leave the brood pouch. Both the extrusion of the eggs and the liberation of the young ones take place at night as will be described later.

* Readings from Subramaniam and Gopala Aiyar, *Proc. Ind. Acad. Sci.*, 1936, 5, No. 5.

Methods

Eggs and embryos were carefully extracted from the brood pouch by means of a pipette and fixed in B G Smith's (1912) fluid for 48 hours. The fixative was prepared fresh every time and was changed after 24 hours. For studying the growth stages the eggs were mostly fixed with the mother and cut *in situ*. They were also dissected out occasionally before fixing. After fixation the material was washed with distilled water and was graded up immediately for sectioning or stored in 5% formalin. Absolute alcohol was not used in dehydration and the embryos were cleared in methyl salicylate from 90%. Paraffin of 58° M P was used for imbedding. About 20 minutes' impregnation was found to be ample. Since the yolk becomes dark by the fixation, leaving the blastoderm unstained, it was not difficult to orientate even the very early stages. Sections were cut seven microns. There was slight fracturing of the yolk, but it was soon found that it could be reduced to a minimum by dehydrating the material in as short a time as possible. Sections were stained mainly with iron hæmatoxylin. Mallory's triple stain did not give good differential staining as Manton (1928) says. For the surface study of the blastoderm embryos were fixed in sublimate and, after removing the egg membranes, were stained with Delafield or Heidenhain, and mounted whole with the ventral side up. It was possible to dissect off the blastoderm in the very early stages, but with the later stages the attempts failed.

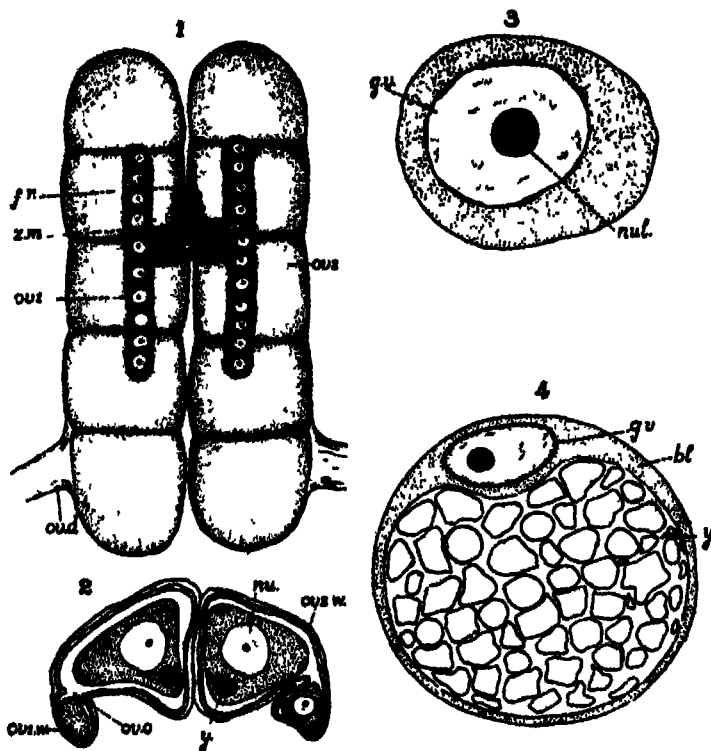
The Female Reproductive System

Our knowledge of the anatomy of the reproductive system of *Mysis* seems to be based entirely on the classical investigations of G O Sars. Though the main features of the anatomy have been brought to light by him, details are lacking. Since a knowledge of these facts is necessary for an understanding of the reproductive processes, a brief account of the female genital organs is given here. Moreover *Mesopodopsis orientalis* presents features which appear in some respects to be new.

The ovary (Fig 1) is made up of a middle piece and two pairs of parallel tubes lying on either side of it. It is situated in the interspace between the alimentary canal and the pericardial floor in the dorsal side of the body. The two tubes on either side lie one above the other and the upper one is much broader and longer than the lower. The bridge-like middle piece is attached to the lower tubes half-way along their length. At the anterior end the lower tubes open into the upper by two fairly wide apertures (Fig 2). In his figures of the ovary of *Mysis oculata*, Sars (Zimmer, 1927) does not show such a lower tube. There the lateral extremities of the middle piece

are shown to bend forwards slightly and lead into two parallel tubes which are directly connected with the oviducts. But in *Mesopodopsis* the eggs first pass into a pair of lower and narrower tubes, where they rest for some time and undergo part of their initial growth and then only enter the large upper tubes. The oviducts are given off from the outer angles of these upper tubes a short distance in front of their posterior end. They travel outwards and downwards between the pericardial floor and the thick longitudinal muscles and get considerably compressed in the process. Below the ventral longitudinal muscle each oviduct bends inwards and opens into the brood pouch on the top of a small backwardly directed prominence from the base of the sixth thoracic leg. At the distal end the duct gets slightly dilated. The external opening lies collapsed usually.

The bridge-like middle piece is the real formative region of the ovary. It is thin and solid with a delicate tight fitting covering membrane, showing small nuclei, and lies slightly depressed in the middle. Three distinct regions could be made out in this part of the ovary. (1) The apex of the triangular promontory, which shows small oogonia packed closely and pressed into polygonal shapes. The nuclei are round, each with a nucleolus. (2) A middle zone consisting of large cells arranged in a row which starts from the centre of the triangle and travels outwards on either side along the anterior margin of the bridge. In these the bigger cells are found towards the outside and the others are arranged in regular progression behind. (3) The region lying behind the above zone along the posterior margin of the bridge and consisting of elongated closely packed transversely placed cells. Their nuclei are elongated and differ very much in appearance from those of the cells in the two anterior zones. The cytoplasm of these cells are drawn out at the ends which are wedged into the interspaces of adjacent cells. This shows that the region consists of tissue which gives support to the germinal cells. The wall of both the ovarian tubes is continuous, showing very small nuclei and is invested with a thin connective tissue membrane which in sectional preparations is often torn apart from it. The opening between the two tubes is not provided with any valvular or muscular structures and nowhere along their course do the tubes show any glandular modification of their cells. As the eggs inside the tubes enlarge the wall of the latter is stretched considerably and in the female pregnant with ripe ova it appears like a thin membrane with sparsely distributed nuclei. Due to the high tension, the inter-nuclear regions of the wall become extremely drawn out. This happens both in the upper and the lower tubes. After the extrusion of the eggs the upper tubes shrink to a fraction of their previous dimension and consequently the wall becomes thick and closely nucleated.



FIGS 1-4

The oviducts are given off almost at right angles to the longitudinal axis of the ovary and their walls are as thick as the shrunk upper tubes, with larger nuclei. At the base its roof and floor come close to each other and almost block the passage. The inner ends of the big cells of the entire oviduct are very uneven and this becomes more and more marked as we come down its course and reaches the maximum in the distal swollen portion. This region of the oviduct is very glandular and shows large cells with deeply staining nuclei. During the time of breeding the glands become suddenly active and produce a secretion which fills the whole oviduct in the form of a bundle of fine long threads. Once the eggs are extruded the oviduct becomes empty and its walls collapse. No more secretion is produced till the next breeding.

The Egg

The youngest eggs are found in the triangular promontory of the middle piece of the ovary. Towards the second region of the middle piece the appearance of the nucleus totally changes. It becomes a clear circular space and a prominent nucleolus makes its appearance in the centre of it. The

eggs pass along the middle zone into the centre of the lower tube of the ovary where they get themselves arranged one behind the other. Eight to ten or twelve eggs could be counted in each tube. When the eggs first enter the lower tube each one measures from thirty to forty microns in diameter and they remain there till the size is doubled. Due to mutual pressure the posterior and the anterior sides of the eggs become flat as they enlarge. At this stage the cytoplasm is lightly stained by hæmatoxylin and shows no yolk inclusions. The nucleus also enlarges considerably, measuring about 35 microns (the nucleolus about 15), and remains clear and prominent as before exactly in the centre of the cytoplasm (Fig 3). In addition to the large nucleolus small circular bodies, highly basophilic in character, could be noticed frequently in the nucleus.

As the upper tube becomes empty by the extrusion of the previous crop of eggs into the brood pouch, the smaller ones enter it one after another through the opening at the anterior end of the lower tube and arrange themselves in a row. As has been said above, the walls of the upper tube are very much shrunk after extrusion and the fresh eggs which enter it do not get space enough and consequently press against them. Immediately after entering the upper tube, yolk begins to be secreted inside the egg. The first globule of yolk makes its appearance in the lower region of the egg and could be seen in sections as a yellow circular patch. Further deposition of yolk takes place at the same spot and on account of this localized deposition the nucleus of the egg loses its central position and is shifted to the periphery on the opposite side. Yolk formation continues and the cytoplasm is stretched out into a thin rind around the yolk with a thicker region on the upper side where the germinal vesicle comes to be situated. The latter retains its original appearance of a clear circular space with a prominent nucleolus in the centre. At this stage the egg looks like a signet ring in cross-section (Fig 4). The lateral expansion of the ovarian tube is prevented by the body wall and the liver tubes. Consequently they are pressed into oblong shapes and could be seen projecting much beyond the limits of the lower tube at the anterior end and the points of origin of the oviducts at the posterior. In the living animal the two rows of eggs could be seen through the transparent body wall. The yolk spherules glisten brightly and if examined under the microscope the superficial germinal vesicle could be seen on the upper side of the egg. It is very difficult to dissect out the eggs at this stage, for the wall of the dorsal tube, stretched to its utmost, bursts at the slightest touch and lets out the semi-fluid egg masses. In the living condition the yolk is colourless and the egg measures about 375×320 microns. Inside the ovarian tube the eggs do not possess any definite

covering membrane The number of eggs varies ; usually in each tube there will be four or five and the number in the two is almost in all cases equal.

The changes accompanying the growth of the egg of *Mesopodopsis* inside the ovary just now described are very remarkable All Crustacean eggs are believed to be centrolecithal (MacBride, 1914, p 177) and in this condition the nucleus lies together with an accumulation of protoplasm near the centre of the egg Probably the only exception to this is the egg of *Lepas*, described by Bigelow (1902), where soon after the extrusion of the second polar body an elongation sets in and the yolk is pushed to the vegetal pole, thereby rendering the egg telolecithal Rarely, however, it has been noted that the first nucleus is found not exactly in the centre of the egg but in an eccentric position somewhat nearer the animal pole [e.g., *Moina*, *Cetochilus*, Korschelt and Heider (1899), *Jasus lalandi*, Von Bonde (1936)] The typical centrolecithal condition is brought about by the uniform deposition of yolk all round the germinal vesicle In *Mesopodopsis* on the other hand, the deposition of yolk is confined to one side of the germinal vesicle which is consequently pushed to the surface Because of this the polarity of the egg is determined very early in its growth and the ripe egg is rendered telolecithal In this connection it may be pointed out that the terms centrolecithal and telolecithal, though constantly used, are rather ambiguous and a clear definition of them is highly desirable The author was reluctant to describe the fully grown egg of *Mesopodopsis*, prior to maturation, as telolecithal because, the disposition of yolk and the germinal vesicle in the eggs of the species seemed to be only an exaggeration of the condition that is said to prevail in *Moina*, *Cetochilus* and *Jasus* But accidentally it was found that Smith (1912) has described a somewhat similar condition in the growth of the egg of the amphibian *Cryptobranchus*, and has characterised the fully grown egg as telolecithal The only difference that I could find between the two eggs is that while in *Cryptobranchus* the peripheral layer of cytoplasm becomes indistinguishable from the yolk as a separate layer in the vegetal hemisphere, it remains clear and distinct on all sides though much thickened at the animal pole in *Mesopodopsis* But this is of no account, since the presence of cytoplasm—however thin or indistinguishable it may be—around the yolk is necessitated by the fact that the latter is a cytoplasmic inclusion This fact seems to vitiate, at least theoretically, the difference between the terms centrolecithal and telolecithal

Copulation

The growth of the egg, described above, takes about three to four days for completion During the course of the investigation it was observed that invariably the final stages of this were reached towards the evening and that

by the next morning all the eggs inside the body had been extruded into the brood pouch. Repeated attempts to see the act of copulation and extrusion during the daytime proved unsuccessful and so a number of pregnant females and mature males were kept under observation at night. A rectangular aquarium jar with clean flat sides was selected and filled with clear brackish water. It was placed on a raised platform over the table in a level with the eye so that the behaviour of the animals could be watched without strain. Half-a-dozen females showing the biggest eggs and a dozen large robust males were selected and put into the vessel and a powerful light was fixed behind it. They were closely watched for hours together at night and it became possible to observe their reproductive processes.

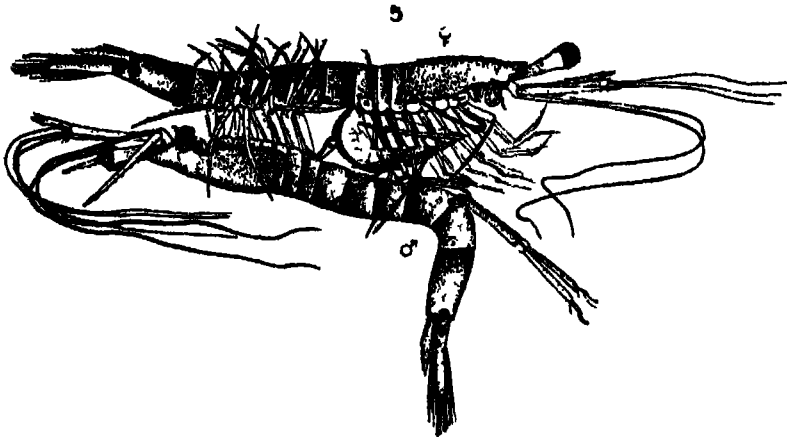


FIG. 5

The animals were freely swimming for a long time without any unusual behaviour either on the part of the females or the males and the first thing observed was the moulting of the female specimens. This happened between 10 and 11 P.M. In the course of this one hour all the six females moulted. Just before moulting the animal exhibits a clumsy and very tired appearance and ceases to swim actively. It then reels to the bottom of the jar as if utterly fatigued. A minute later it jumps up again, but falls down and lies on its back. In this posture it bends the middle of the body and jerks up. By three or four such sudden jerks it frees itself from the moult and tries to swim. All this is over in three minutes' time, but the animal is too weak to swim and limps at the bottom of the jar. It could be seen suddenly jerking up, and then, unable to swim, falling by its own weight. This kind of movement is very characteristic of a freshly moulted specimen. The moult falls off in one piece and it shows a longitudinal cleft on its ventral

side After some five minutes of struggling the animal gains strength and begins to swim about actively In this state the body wall is unusually transparent and by examining the whole animal under the microscope the shape and working of many of the internal organs could be studied. On catching sight of a freshly moulted female the male comes near and begins to follow it Neither sex exhibits any frantic or impatient movements, but proceeds leisurely After about five minutes of patient chasing, the male commences to rub his antennæ on the sides of the female and tries to catch hold of the posterior end of her body by his anterior thoracic legs When he succeeds, arching the last abdominal segments upwards, he suddenly swings down and brings his body below that of the female, the ventral sides of the two facing each other in the act, and ejects the sperms into her brood pouch As the whole process is very quick it was not possible to observe the actual ejection of sperms The animals do not remain united even for one minute After one sudden jerk the male swings-back but the process is repeated three or four times in quick succession In all cases the brood pouch of the female when examined immediately after the process showed large numbers of spermatozoa Of the six female specimens kept in the dish during the experiment only four were observed to copulate and only one male was observed to be involved in each case The other two females though they were not seen to copulate during the course of the experiment extruded the eggs and developed young ones In a similar way a set of four females isolated and kept in a dish without any male specimens developed broods in the normal manner. Probably the sperms necessary for fertilization in these cases were present in the brood pouches of the animals as a result of recent copulation

Extrusion of the Egg

As will be clear from the foregoing section extrusion of the eggs may or may not be preceded immediately by copulation, but it has invariably been observed that the female moults before the process A similar behaviour is recorded in the fresh-water Macrurous Crustacean *Atyephyra compressa* by Ischikawa (1885) and in several species of *Gammarus* by Della Vale (Korschelt and Heider, 1889), and also in *Jasus* by Von Bonde (1936). As extrusion also takes place only at night, experiments similar to the one described above had to be arranged to observe the process Besides the ecdysis of the mother, the size and appearance of the eggs are sure indications of their approaching extrusion. After a little experience one can easily say when a particular specimen will extrude its eggs. Under the microscope this is easier still, for, just before extrusion the eggs show some unmistakable symptoms The large cup-shaped germinal vesicle, so prominent before the

moult on the dorsal side of the egg slowly breaks down. With the breaking down of the germinal vesicle the protoplasmic thickening in which it was situated also shrinks considerably and almost reaches the level of the layer round the other sides of the egg. At this stage no trace of the nucleus could be seen anywhere in the living egg. The eggs are now ready for extrusion. The animal becomes lethargic in its movements and prefers to float quietly in the water rather than swim. It shows frequent wriggling movements, bending and straightening its body in the vertical plane. As a result of this the eggs slowly drop into the brood pouch. The details of this could be observed under the microscope by keeping an animal in water on a slide. The oviducts are narrow and they dilate but little, hence the eggs cannot pass through them without altering their shape. Consequently the eggs are seen to flow out gently through them in a narrow stream into the brood pouch. It takes from 30 to 40 seconds for a single egg to ooze out in this manner. The basal portion of the oviduct is found to dilate more than the distal. The hindermost eggs are the first to come out and the others follow in regular succession with practically no interval. Extrusion is simultaneous on the two sides, but when one row contains more ova than the other, as sometimes happens, the animal has been found to struggle hard to get rid of the last eggs. The globulated consistency of the eggs is clear even when they are flowing out.

As has already been stated the eggs are naked while inside the ovarian tube. But as they flow down the oviduct and reach the genital pore a thin membranous covering makes its appearance. This membrane is the first to come out of the genital pore where it remains for a while like a bubble (Fig 44). It assumes the same size as the egg while the latter is still in the oviduct, and is filled with fluid. The egg collects into a globule at the oviducal orifice and drops to the bottom of the membranous vesicle. The membranous covering remains attached to the genital pore till the next egg comes out and pushes it back into the brood pouch. All these membranes stick together and remain attached to one another. After falling into the brood pouch the eggs assume a spherical shape and measure on an average 350 microns in diameter. The envelope is much larger, measuring 500 to 600 microns and remains distended. In sections the interspace between the egg and the covering membrane is found to contain a finely granular substance which is stained by basic dyes. After the extrusion of the eggs the ovarian walls collapse and the secretion contained in the oviduct disappears.

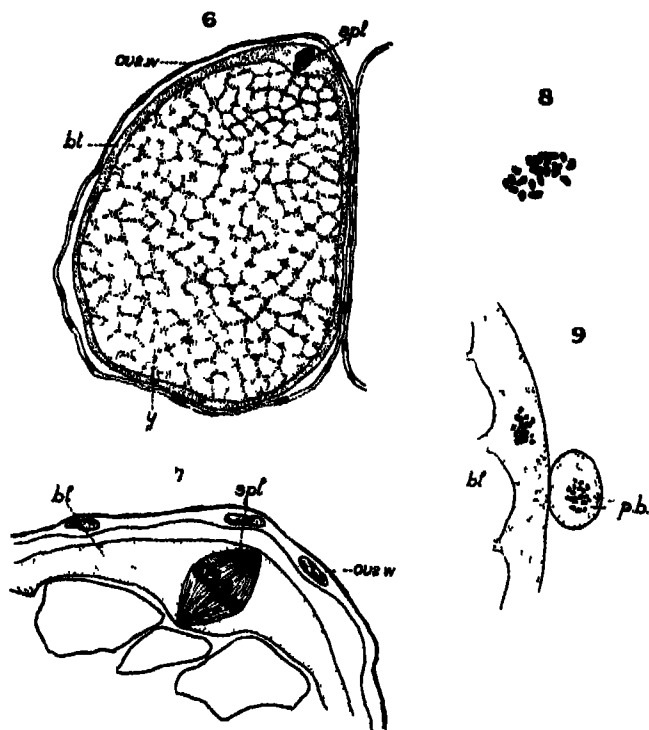


FIGURE 6-9

Maturation and Fertilization

The breaking down of the germinal vesicle mentioned above evidently marks the termination of the growth period of the egg and the onset of the processes of maturation and reduction. In this connection it has to be pointed out that chromosome studies in the gametes of the Crustacea are not many in number. Among the Entomostraca, the Copepoda and Phyllopoda have been subjected to intensive studies in this direction, while among the Malacostraca the Decapoda alone seems to have received fair attention. Even among these one finds that the majority of the papers deal only with spermatogenesis and leave the origin, growth and maturation of the egg obscure. From the literature available I find that nothing is known about the oogenesis of the Schizopoda and consequently the pre-zygote history of the egg of a Mysid Crustacean is being described here, I believe, for the first time. In spite of the large quantity of yolk present in the egg, the small size of the division nucleus, and the rapidity with which the polar divisions take place, it has fortunately been possible to trace the oogenesis of *Mesopodopsis* in broad outline. The polar divisions do not take more than 15 to

20 minutes for completion and fertilization follows immediately. Only a fraction of this time is spent inside the brood pouch and so to get all the stages several specimens just before, while engaged in, and immediately after extruding the eggs were suddenly dropped into the fixative and carefully sectioned.

Before the wall of the germinal vesicle breaks down certain marked changes are seen in the nucleoplasm. The large prominent nucleolus slowly dissolves away leaving no trace of its existence behind. It does not break up into bits and get scattered in the nucleoplasm as happens in many cases (see Montgomery, 1899), but remains condensed and fades out centripetally. This is accompanied by the appearance in the nucleoplasm of lamp brush like aggregations of chromatin, zig-zag in shape and exhibiting increasing basophily. Outside the germinal vesicle the layer of cytoplasm surrounding it becomes thinner and thinner and prolongations from it penetrate into the yolk mass below. Very soon the nuclear wall breaks up, the nucleoplasm mingles with the general cytoplasm of the egg and the first polar spindle is formed. The spindles are very small, 20 to 22 microns in length and 12 to 13 microns in breadth and are formed in the inner dorsal angles of the eggs. The majority of them lie in a vertical or transversely horizontal plane, but all sorts of oblique positions are not uncommon. As stated before, the breaking down of the germinal vesicle is followed by an immediate shrinkage of the mass of cytoplasm in the region and when the spindle is formed the space available appears too narrow for it. Consequently its inner ends actually verge on the yolk mass (Figs 14 and 15). This spindle is typically anastral showing no astral rays and central bodies, though at either pole the fibres converge to a single point. It cannot be called barrel-shaped as Wilson (1925) characterises all anastral spindles, but the central portion is found to be swollen a bit disproportionately.

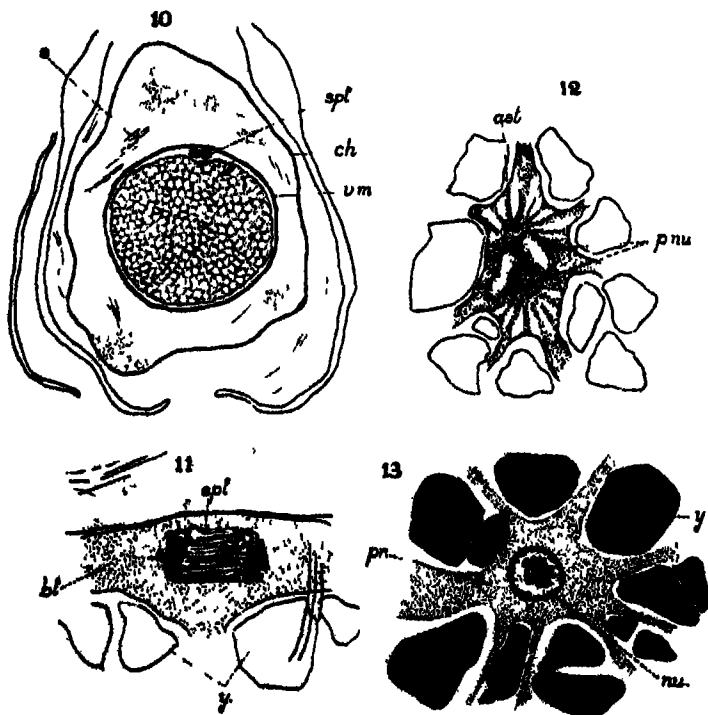
The presence of anastral spindles in polar mitosis has been demonstrated in several animals and the problem of their nature and origin has engaged the attention of many students of Cytology. Regarding their origin the view is held that in the oocytes of certain animals they arise "wholly from the nucleus without visible participation of the cytoplasm" (Wilson, 1925). In the present study it has not been possible to investigate the origin of the maturation spindle and the author is unable to draw any conclusion on the above point. It should, however, be pointed out that the spindle just now described is very small when compared with the germinal vesicle which precedes it—its length forming hardly a third of the diameter of the latter as would already have been noted from the measurements given. And also that the wall of the germinal vesicle has been found to break up

prior to the appearance of the maturation figure causing, as it will, the mingling of the nucleoplasm and the general cytoplasm. In the face of these facts the view that there is no participation of the cytoplasm in the formation of the spindle requires further support. There is an additional interest attached to the presence of anastral spindles in the oogenesis of *Mesopodopsis*, since, as will be shown in the course of the paper, the segmentation spindles of its developing eggs are typically amphiastral, characterised by sharply marked astral rays and central bodies. The chromosomes are small and dot-like, but in the metaphase of the first polar spindle they appear like dumb-bell shaped bodies arranged equatorially. Though several attempts were made their size was found to be too small for a detailed study of the internal phenomena of meiosis. The haploid number of chromosomes as counted from several meiotic figures and also from the resulting polar bodies is 22 (Fig. 8). It will be shown in the following pages that the diploid number is just double this.

While the nucleus is in the metaphase of the first polar division the egg is extruded into the brood pouch and in the process a membranous covering appears around it. Much confusion still exists regarding the number, origin and terminology of the envelopes of the Crustacean egg. McMurrich (1895) found two envelopes in the eggs of *Jæra*, *Porcellio*, *Asellus* and other Isopods which he studied and says, "it seems to be a very general rule that the ova of the Crustacea when extruded are enclosed within a single membrane, the chorion, which is almost certainly formed by the secretions of the follicular cells, though some authors have referred its formation to the walls of the oviduct." But the same author recognized a few exceptions to this. Cases were on record even then and these were stressed by Korschelt and Heider (1899), where the eggs were observed to pass into the brood cavities without an external envelope. More recently Cannon (1921) has shown that the summer eggs of *Simocephalus* are extruded in the same fashion and secrete a covering only later. The membrane which invests the egg of *Mesopodopsis* as it extrudes into the brood pouch is not homogeneous in structure and shows several laminations which are often loosely attached to one another. The whole process of extrusion has been carefully observed and it is found that the egg when it enters the oviduct is naked and uncovered, but acquires the aforesaid envelope as soon as it reaches the genital pore. This fact and the histology of the oviduct leads me to conclude that the covering membrane is secreted by the epithelial cells in the lower portions of the oviduct. The large size of the membrane compared with the egg, its close adherence to the epithelial cells in the process of extrusion and its laminated structure also point to the same conclusion. There can be

no doubt therefore that this envelope of the egg is the chorion. There is no record in the literature available to me of its presence in other Mysids. It has not been mentioned in connection with the eggs of *Mysis* (Nusbaum, 1887 and Bergh, 1893) and in *Hemimysis* Manton (1928) definitely says that there is no chorion. McMurrich (1895) quotes several authorities to prove its almost universal occurrence in the Decapoda and more examples have been provided recently by the work of Von Bonde (1936) and Zehnder (1934) on *Jasus* and *Asiacus* respectively. But regarding the other groups of the Crustacea the evidence seems to weigh in favour of its absence. Della Vale (cited by Korschelt and Heider, 1899) has shown that in *Gammarus* the eggs are passed into the brood pouch without an external envelope. Nor does Manton (1934) speak of a chorion in the eggs of *Nebalia*.

The first polar division is completed soon after the egg reaches the brood cavity and a very small polar body, measuring 10-12 microns is extruded (Figs 9 and 45). Just when this polar body is getting rounded off from the egg it is possible to count the number of chromosomes in it, for they remain separate and free from one another. But very soon the chromosomes fuse together to form an irregular network and the polocyte dwindles away. It has



FIGS. 10-13

never been found to divide into two before doing so. Only in one instance was it observed to persist, though in a much shrivelled up condition till the first cleavage, but usually it disappears even before the second polar division.

The second polar division follows quickly, but there is reason to believe that before the process is completed the sperm enters the egg (Fig 10). Fig 10 shows the second polar division in the late anaphase. The egg shown was fixed with the mother about ten minutes after it was extruded into the brood pouch and was cut *in situ*. The mother was one of the experimental females which was found to copulate before the process of extrusion. In the section remnants of several sperms could be seen, some inside the chorion, some outside it, while a few are actually in the egg. An important feature of the egg is that the vitelline membrane or the fertilization membrane is already formed (Figs 10, 11 and 47). This is very thin and is separated from the egg by a short distance. It is an established fact now (Wilson, 1925) that the separation of the fertilization membrane from the egg is brought about by the entrance of the sperm. In the section of the egg shown I have not been able to find out the sperm nucleus anywhere, but the presence of sperm fragments and the separation of the fertilization membrane are proofs enough that the sperm has come in contact with the egg. McMurrich (1895) maintained that the vitelline membrane is formed only at the instant of fertilization, but the long series of recent work on the subject seems to favour the view that the membrane pre-exists as such before fertilization. In *Mesopodopsis* it becomes visible only when it is separated. It has not been possible to see the second polar body, and I seriously doubt whether it is extruded at all. In a single instance at a later stage showing the female pro-nucleus, a mass of chromatin was found dwindling away in the peripheral layer of protoplasm. In all probability this is the second polar nucleus degenerating.

The next stage obtained is shown in Figs 12 and 48. This is a happy preparation showing the two pronuclei uniting in the process of fertilization. They are situated in an island of protoplasm near the centre of the egg and hence it follows that after the liberation of the polocytes the female pronucleus sinks into the depths of the yolk. Unfortunately the author failed to get any intermediate stage showing the pronucleus in the course of this migration from the surface to the interior and the male nucleus approaching it. But the process seems to be very common. Among the Crustacea it has been described in several species of *Cyclops* (Wilson, 1925) and also in the stone crab *Mesippe mercenaria* (Binford, 1913), to take only two cases. It seems to be a very common phenomenon in the insects and to cite a recent example, Inkmann (1933) has shown that in the corn weevil *Calandra granaria* an identical process takes place during fertilization. In *Mesopodopsis* apparently

both the pronuclei have to travel almost the same distance, since the female pronucleus also occupies an extreme superficial position originally and since the meeting place of the two has been shown to be central. In the figure obtained the pronuclei show a vesicular structure and are so identical in form and size that it is impossible to distinguish the male from the female. Two large and vaguely spreading asters could be seen on either side of the nuclear couple, but they are not clear enough to be described in detail.

Having met, the two pronuclei fuse together and form the cleavage nucleus shown in Fig 13. The star-shaped island of protoplasm of the previous stage persists and measures about 18 to 20 microns across at its maximum. The prolongations from this go far out into the yolk and most of them get attached to the peripheral layer of protoplasm. The latter though progressively becoming thinner and thinner, is still clear and measures on the average 5 to 6 microns in thickness. The egg now agrees with the one-celled stage figured by Boutchinsky (1890) for *Parapodopsis cornuta*. Even in *Mysis* where van Beneden (1869) and Nusbaum (1887) maintained that purely discoidal cleavage takes place, the cleavage nucleus occupies a similar position originally. Manton (1928) was not able to get the one-celled stage in *Hemimysis*, but from the succeeding stages and the nature of segmentation rightly infers that the cleavage nucleus is situated centrally in the egg.

It will be seen from the above account that the whole series of nuclear changes connected with the phenomena of maturation and reduction in *Mesopodopsis* take place in the thin surface layer of protoplasm. All these processes, as also the subsequent fertilization follow in quick succession. Only a very small fraction of the eggs fixed during daytime from early morning collections showed even the late segmentation stages. Ninety-five per cent of the eggs would have already formed the blastodisk and will be well advanced in the differentiation of germ layers by the time they are fixed. This probably explains the failure of Manton (1928) to study the very early segmentation stages of *Hemimysis* and the oogenesis of *Nebalia* (1934). It has been found in most cases that in less than an hour after the breaking down of the germinal vesicle the egg is well advanced in segmentation. After the second polar division the female pronucleus sinks into the interior of the yolk, as was shown above, and this results in making the egg typically centrolecithal. Thus it may be said that from its origin till the establishment of the cleavage nucleus the egg of *Mesopodopsis* passes from an alecithal, through a telolecithal to a centrolecithal condition.

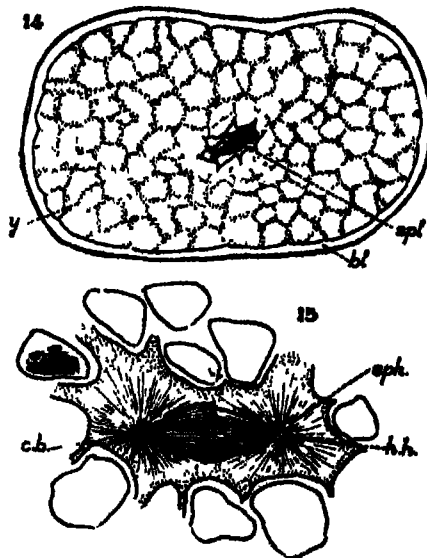
Segmentation

In studying the segmentation of *Mesopodopsis* it has not been possible to trace the cell lineage even in broad outline, because of the small size of the

egg, the opacity of the yolk and the minuteness of the cleavage nuclei. In the living egg, though the yolk is unpigmented, the nuclei could not be made out and fixation and subsequent clearing in oils did not help matters. The following account is therefore based on facts gathered from serial sections of different stages of the egg. The fertilized egg is slightly oval in shape and measures about 370 microns along its longer axis. The chorion which in the beginning was distended due to the accumulation of fluid shrinks considerably and lies very close to the vitelline membrane near the periphery of the egg. The yolk appears in fairly large-sized globules and a thin peripheral layer of protoplasm could be distinguished even in the living egg.

The First Cleavage

Figs 14, 15 and 49 show the metaphase spindle of the first cleavage. The plane of cleavage in relation to the polarity of the egg could not be determined, since the polar body has dwindled off and has left no mark of its position in the sections. But by reconstructions from serial sections of another egg of a slightly later stage where the division is completed and which shows a very much shrivelled up polar body on one side, I find that the plane of the



FIGS 14-15

first cleavage is almost at right angles to the polar axis of the egg, but as I have observed it only in a single case, the fact is not pressed here for want of more proof. It will be seen from the figures that the star-shaped island of protoplasm at the centre has been stretched out by the spindle which measures 30 microns from pole to pole and 10 microns across the equatorial plate, where

it almost touches the limit of the protoplasm. In the section the yolk appears as polyhedral masses, leaving interspaces through which, occasionally, protoplasmic strands could be traced reaching out into the peripheral layer of protoplasm from the central island. In the equatorial plate of the division figure 44 dot-like chromosomes have been counted and this fixes the diploid number at twice the gametic, given previously. The achromatic figure is of the typical amphiastral type, described in the cleavage of several animals and shows a clear and conspicuous central apparatus at either pole. The latter consists of two minute and closely lying central bodies,* surrounded by a heller Hof or clear area, which again is surrounded by a prominent sphere showing sharply marked astral rays (Figs 15 and 49). There is absolutely no mark of this cleavage on the egg as a whole, both the yolk and the blastema remaining unaffected. The two daughter nuclei formed by the first division separate with a small amount of protoplasm, but do not wander far away from each other.

The second division has not been observed, but a later stage showing four blastomeres has been obtained. One of these is shown in Fig 50. Here also the blastomeres do not drift away but remain much nearer the centre than the surface of the egg. Each blastomere consists of a small radiate mass of protoplasm containing a nucleus which almost fills it. By this time the peripheral layer of protoplasm becomes extremely thin and indistinguishable from the yolk. Manton (1928) says that this layer remains clear at the animal pole of the egg throughout the period of segmentation, but I have not been able to observe this in *Mesopodopsis*. Here, soon after cleavage begins the blastema thins down on all sides and nowhere could it be distinguished as a distinct layer outside the yolk. As segmentation proceeds the blastomeres progressively lose their amœboid character and get themselves rounded off. This change is especially marked towards one side of the egg and thus as judged from succeeding stages is the animal pole. The change in the shape of the blastomeres is accompanied by a remarkable arrangement of their position. It was said before that after division the blastomeres do not wander away much from the centre of the egg. This behaviour continues until approximately at the 128 celled stage they arrange themselves to form a spherical vesicle, one cell thick all round and lying deep within the yolk (Fig 51). The blastomeres lying towards the animal pole are large and spheroidal, while those on the other sides are long and drawn out and are arranged end to end. All the cells are characterised by rounded nuclei.

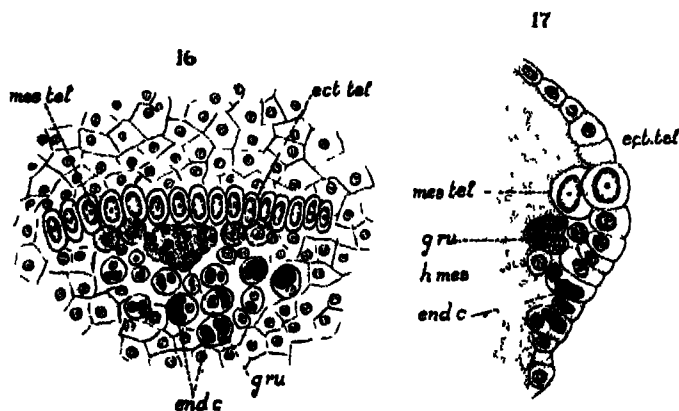
* Much confusion exists in the descriptions of the central apparatus and I here adopt the terminology given by R. H. Bowen in his paper "The Golgi Apparatus—Its Structure and Functional Significance," *Anal. Rec.*, 1926, 32, No. 2, 165.

None of the blastomeres composing the vesicle has been found to divide after this arrangement has taken place, and hence, as the vesicle rises bodily to the surface, much in the manner of a bubble coming up from under water, the layer of cells becomes stretched out considerably on all sides except the animal pole where the cells more or less retain their original spheroidal form. It cannot be said that one particular side of the vesicle reaches the surface earlier than another, for I have not been able to see any definite orientation in its appearance

The segmentation of the egg and the formation of the blastoderm, just now described, are similar to the same processes described by Manton (1928) for *Hemimysis*. It is therefore beyond doubt that in these two forms the cleavage is of the superficial type—Type III of Korschelt and Heider (1899), with this difference that the blastomeres do not rise to the surface individually as in *Astacus* (Morrin, 1886, cited by MacBride, 1914) and other forms, but in a body. In *Mesopodopsis* this latter feature is very much more pronounced than in *Hemimysis*, so much so that the blastoderm can be considered to be formed actually within the yolk itself. A simple superficial type of cleavage has also been recorded in *Parapodopsis cornuta* by Boutchinsky (1890). But the fact remains that van Beneden (1869) and Nusbaum (1887) have shown that in *Mysis* cleavage is of the truly discoidal type and have also given figures of the zygote nucleus dividing at the top of the egg to give rise to a cap of cells. As early as 1899 Korschelt and Heider pointed out the anomaly of this case compared to other Crustacea and suggested that the cleavage of *Mysis* might in reality be an extreme case of superficial cleavage defined as Type III *b* by them. They have even raised the doubt whether true discoidal cleavage ever occurs in the Crustacea at all. Now that superficial cleavage has been shown to occur in the two forms *Hemimysis* and *Mesopodopsis* other than *Parapodopsis*, already described by Boutchinsky, the contention of van Beneden and Nusbaum regarding the segmentation of *Mysis* becomes questionable and the early embryology of the form merits careful reinvestigation. Compared with *Hemimysis* the form here described differs mainly in the formation of the blastodermic vesicle deep down in the yolk and the lack of regularity in its appearance at the surface. From Manton's figures it is seen that in *Hemimysis*, by the time the blastomeres arrange themselves into a layer most of them are very near the surface, and some, at the vegetal pole, have actually reached it. Regarding the appearance of the blastomeres at the surface Manton finds that they first rise at the vegetal pole. Perhaps the behaviour of *Mesopodopsis* shows that much significance need not be attached to this point.

Gastrulation and Establishment of Germ-Layers

After rising to the surface the blastomeres aggregate near the future ventral surface of the embryo and form the undifferentiated blastodisk in which, very soon, the germ elements become marked out. Fig 16 is drawn from a whole mount of the blastoderm and shows the superficial differentiation of, and the spatial relations between the germ elements. From preparations of the preceding stages it is found that the straight row of ectodermal teloblasts is formed by the union of two half-rows on either side, which were initially curved backwards. The number of cells in the full row varies from 14 to 16 and they are formed by the modification of ordinary blastoderm cells, as was observed by Bergh (1893) in *Mysis*. The genital rudiment characterised by small, deeply staining nuclei lies in the median line, just behind the ectodermal teloblasts, and on either side of it a few mesoderm cells are getting differentiated. The endoderm cells, heavily laden with yolk, are lying hindermost. The process of gastrulation by immigration of cells from the area lying immediately behind the row of ectodermal teloblasts is seen in the para-sagittal section shown in Fig 17. The blastopore is represented by the small



FIGS 16-17

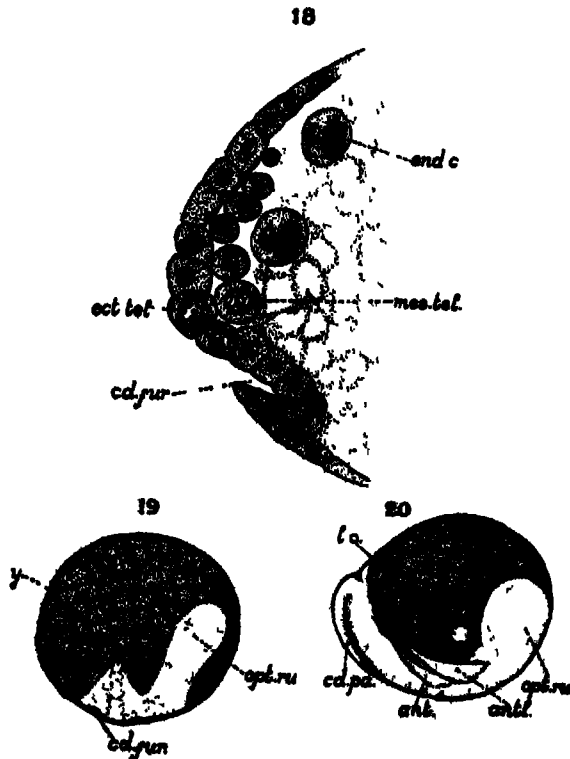
depression at the place where the cells are sinking in. Eight of the inwandered mesoderm cells arrange themselves in a row to form the mesodermal teloblasts, one of which is shown in Fig 17. A portion of the genital rudiment which is slowly moving forwards is also shown in the same section. Close behind it are seen a few cells, resembling the mesodermal teloblasts in appearance, but smaller in size. These are evidently the head mesoderm cells. The endoderm cells are seen in the process of immigration from the surface into the interior. Fig 30 shows a much later stage where the endoderm cells are

found to engulf more and more yolk and spread into a layer round the yolk-mass below the ectoderm

Developmental Stages and Organogeny

Stage I —

Here the blastoderm appears as a V-shaped band on the ventral side (Fig 19). The germ layers are well established and the teloblasts have begun to divide actively. The head mesoderm cells have moved forwards below the genital rudiment and have arranged themselves in three paired bands to form the mesoderm of the naupliar segments. The naupliar appendages have not yet appeared. The ectodermal teloblasts stand out from the ventral surface and have given rise to two rows of descendants. The caudal furrow is being formed immediately *behind* the row of ectodermal teloblasts. In Fig 18 it has deepened considerably. From sections it appears to be formed by a deepening of the blastoporal depression. Gastrulation ceases immediately the first division of the ectodermal and mesodermal teloblasts is over and no more immigration of cells from the area behind the teloblastic row takes place.



Figs. 18-20

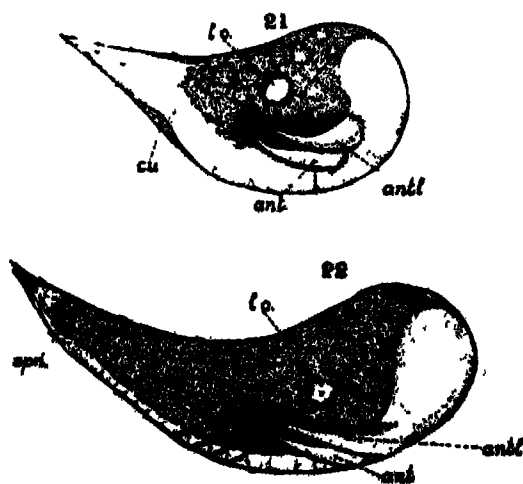
Stage II —

Fig 20 This stage falls just before hatching takes place The embryo is still within the egg membranes The caudal furrow is complete and the papilla, measuring about 175 microns from its base to the tip, stands closely pressed against the vitelline membrane Its base forms a projection at the hinder end The antennule and the antenna are well developed and lie closely applied to the body due to the pressure of the enveloping membranes They are now long ectodermal tubes tapering to fine points and are almost of the same length as the caudal papilla The mandibles are not developed as projecting appendages like the antennule and the antenna The paired lateral organs are well developed and appear as two small disks on either side The germinal band forms only a small strip on the ventral side and the bulk of the embryo is occupied by yolk From sections it is found that 14 mesodermal somites beginning with the antennulary are formed at this stage (Fig 27) The teloblasts occur a short distance in front of the angle of the caudal furrow (Fig 26) and not on the ventral side of the caudal papilla as in *Hemimysis* (Manton, 1928, pl 21, Fig 6) In Fig 27 the mesodermal teloblast is seen dividing to form the fourteenth somite from the antennulary No endoderm cells are found to wander into the yolk from the region lying between the teloblasts and the tip of the caudal papilla The genital rudiment lies between the first and second maxillary somites The yolk cells have spread round the yolk mass to form secondary yolk pyramids In the caudal papilla the whole of the yolk is engulfed in this manner, but at the anterior end a large quantity of yolk remains undisturbed The preantennulary mesoderm is just slipping in at the anterior end (Fig 28) As was said above, the appendages are mere ectodermal tubes and the head mesoderm at their bases is undifferentiated The paired lateral organs measure 50 microns across and 20 microns in depth The cells have lost their boundaries and the cytoplasm has become finely granular The nuclei are large and remain clear In the development of these organs I have not been able to see a cup-like arrangement of cells before they coalesce and lose their walls as Manton (1928) has shown Neither do they form a duct to discharge their products before atrophy

Stage III.—

This stage occurs soon after hatching has taken place (Fig. 21) Length of embryo 700 microns Maximum height 315 microns The embryos hatch out of the egg membranes 24 hours after the eggs are extruded The process of hatching was observed under the microscope by keeping an embryo of appropriate age under water on a slide Just before hatching the size of the embryo increases by 50 to 70 microns in diameter This is probably

brought about by the absorption of water by the yolk as Manton (1934) suggests. The increase in the volume of yolk causes greater tension on the caudal papilla. As a result of this the egg membranes split along the ventral side and the embryo shoots out tail first. In the present stage the embryo is already covered by the first larval cuticle. The whole body is beset with spines which are mere cuticular projections and are longer and more conspicuous towards the posterior end. The caudal papilla has straightened out, but the point where it was folded forwards is distinctly marked by a constriction (Fig 21). Here the cuticle stands away from the surface of the body unlike in other regions. The portion of the body that lies behind the constriction tapers to a point. The yolk is accumulated in the head region where the optic lobes have approximated themselves. The antennules and antennæ stand out from the body and are bent sideways and backwards with the tip pointed up. They are long tapering tubes of ectoderm standing on a broad base and both the limbs are of the same size. Even at this stage the mandibles are not developed in the form of tubular appendages and are represented only by a pair of prominences behind the bases of the antennæ. Inside the body wall all the thoracic and four of the abdominal somites have been formed. The teloblasts, ectodermal and mesodermal, are still larger than their descendants. The region lying behind the limits of the ectodermal teloblasts possesses only a thin covering of cells, but inside, the endoderm cells form clear secondary yolk pyramids. At the tip a few cells form a cone, the cuticle covering which is studded with three long spines (Fig 31). No caudal furca is present. The stomodæum is not yet formed and there is no indication of the place where it is going to be invaginated. The genital



Figs. 21-22

rudiment remains quiescent in the same position as before. The head mesoderm in the antennular segment has just begun to descend into the appendages while in the next two segments it remains undifferentiated either into the antennal gland mesoderm or the liver rudiment in the mandibular segment. The lateral organs reach the maximum of their activity at this stage.

Stage IV —

Length	900 microns
Maximum height	330 ..
Length of antennule	180 ..
Length of antenna	180 ..

This is approximately 24 hours after hatching. The embryo is bent dorsally with the head very much swollen due to the accumulation of yolk. The posterior end tapers to a point and possesses three long spines (Fig. 31). The antennules and antennae are long and project from the body, but the mandibular palp which is just being formed is kept close to the body by the cuticle and could be clearly seen only in sections. The paired lateral organs are still very active and their secretion products which stain intensely black are visible near the median pore. The ectodermal and mesodermal teloblasts have ceased to divide and seventeen mesodermal somites could be counted even in the living embryo behind the mandibular segment (Fig. 29). The last one evidently corresponds to the seventh embryonic abdominal segment demonstrated by Manton (1928) for *Hemimysis*. In the more anterior thoracic segments, the mesoderm cells have by active division aggregated into two blocks lying ventro-laterally in each segment. But posteriorly they are arranged in rows of eight cells immediately beneath the ventral ectoderm. The first and second maxillae as well as the first thoracic appendages have been formed as buds, which lie bent towards the mid-ventral line inside the cuticular envelope of the embryo. The nerve cord has begun to get differentiated along the middle line on the ventral side from before backwards. The genital rudiment has now shifted further back into the first thoracic segment and becomes divided into two blocks, lying on either side between the ventro-lateral mesoderm blocks and the yolk cells. In the three naupliar segments the head mesoderm shows greater differentiation than the mesoderm of the post-naupliar segments. In the antennular segments it has already begun to form the muscles of the appendages. A portion of the two mesoderm bands here, extend as thin tongues towards the mid-ventral line. No coelomic cavity is formed in the mesoderm of the antennular segment. The mesoderm of the antennal segment is larger than that of the preceding one. It is confined to the ventro-lateral regions of the segment, immediately below the limb bases, and has already formed the antennal gland rudiment. A cluster of cells gets cut off from the mesoderm bands on either side and moves

nearer to the yolk. The nuclei of these cells show evidences of approaching division, though no division figures have been noticed at this stage. The limb mesoderm slowly sinks into the cavity of the antenna. In the mandibular segment the adductor muscles are being laid out and the tendon has been formed. The liver rudiments have differentiated and occur as two blocks on either side of the mid-ventral line above the mandibular tendon. All sides except the base of these mesodermal blocks are surrounded by yolk and the layer of yolk cells could be seen passing over them. The stomodæum has just tucked in immediately in front of the bases of the antennæ. It appears as a very narrow transverse slit on the outside and has got thick walls. The tip of the invagination touches the layer of yolk cells and is slightly bent backwards. The beginnings of a median depression are seen on the dorsal side of the stomodæum. The preantennular mesoderm has extended backwards as a broad one-layered plate on either side (Fig 32). At the hinder end the position of the proctodæal invagination is marked out by the thick crowding together of the ectoderm cells below the seventh abdominal mesodermal somite. In the living embryo the inside of the cone-shaped portion of the body lying behind this is found clear and devoid of the globular structure characteristic of the yolk in the anterior regions of the body. In sections no yolk cells are seen here which shows that all of them have degenerated. The same condition was observed by Manton (1928) in *Hemimysis*.

Stage V —

FIG 23

Length	.	903 microns
Maximum height		315 ..
Length of antennule	.	350 ..
Length of antenna		375 ..

This is about 10 to 12 hours older than Stage IV. The antennule and the antenna have become much longer and show a biramous structure within the cuticle. The mandibular palp is still within the cuticle and is invisible from outside. The labral rudiment is formed. The maxillule, the maxillæ, the eight pairs of thoracic appendages and the rudiments of the abdominal appendages including the uropod have appeared as tubular buds. The seventh abdominal segment is very vestigial and does not develop any appendage. The proctodæum is seen only as a very shallow depression behind the seventh abdominal somite. The stomodæum, on the other hand, has grown further back, its tip reaching as far as the middle of the mandibular segment. In addition to the fold on its dorsal wall described in the previous stage two longitudinal in-tuckings have appeared along its sides. The optic rudiments have become considerably thickened and differentiation of the cell layers is vaguely

beginning The ectoderm has grown in between the optic rudiments, preparatory to the folding off of the latter from the body The yolk septum has made its appearance dividing the anterior yolk mass into two unequal halves from before backwards It is continuous with the body ectoderm and is very thin Manton (1928) says that she was not able to see any nuclei in this structure, but in my preparations I see that it is sparsely nucleated (Fig 36) On the dorsal side of the embryo the median dorsal organ is being formed by the close aggregation of ectodermal cells This process occurs over a fairly wide area, but does not extend deeply The cytoplasm of these cells shows no secretory products as yet The mouth has shifted a little to the back and lies in a line with the hinder edge of the base of the antenna The pair of preantennular mesoderm bands have grown further backwards and now lie in the antennal segment as two triangular masses, with their bases grazing the sides of the stomodæum and the vortices directed forwards A small triangular coelomic cavity has appeared in each mass (Fig 39) In the antennal segment the mesodermal gland rudiment has divided into two, a rounded mass and an elongated band, the latter showing a double row of cells Evidently the rounded mass of cells represents the rudiment of the end sac and the other that of the duct In the mandibular segment the adductor muscles and their tendons have become very massive in consonance with the growth of the mandible itself The liver rudiments have become larger and extend through the whole length of the mandibular segment Their outer edges are curved dorsally and a layer of thinner cells is found to stretch between their inner edges as a bridge (Fig 34) This layer of cells lying in the median line extends forwards and gets attached to the hinder end of the stomodæum Though lying in close proximity to the liver rudiments these cells have an entirely different and belated origin compared with them They are formed by the direct transformation of the layer of yolk cells which originally passed over the liver rudiments, but is now fast breaking up there Undoubtedly this median layer of cells marks the beginning of the midgut epithelium and corresponds to the anterior endoderm plate of Wagner (1898) and Manton (1928) The cells of the liver rudiments are even now large and their cytoplasm has already begun to show a striated appearance The mesoderm of the first maxillary segment is found to be rudimentary and does not develop a coelomic cavity within it But from the next segment onwards the mesoderm bands become more massive There, each ventro-lateral block after giving off the limb mesoderm divides into a dorsal and a ventral mass At this stage of the embryo a definite coelomic cavity is differentiated in the dorsal mesoderm blocks of the following segments.

The maxillary segment.

The first thoracic segment

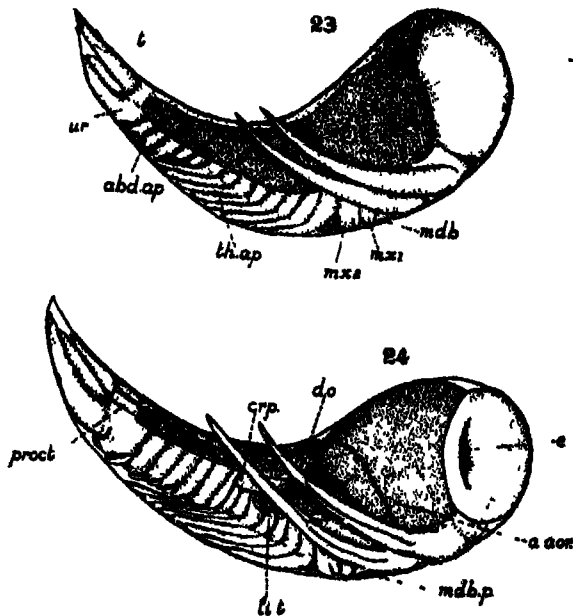
The second thoracic segment.

The third thoracic segment

The fourth thoracic segment

The fifth thoracic segment

Behind the fifth thoracic segment the mesoderm becomes less and less massive till in the vestigial seventh abdominal segment it is represented only by a few cells. The genital rudiments lie just internal to the dorsal mesoderm masses in the first thoracic segment. All the thoracic limbs are formed and the rudiments of the abdominal limbs are also visible. The former are all of the same size and shape and are uniramous.



FIGS 23-24

Stage VI —

FIG 24

Length	..	1187 microns
Maximum height	..	368 "
Length of antennule	.	850 "
Length of antenna		385 "

The embryo is still within the first larval integument which has become considerably stretched due to the increased size of the embryo. The antennule and the antenna have become distinctly biramous, but the rami lie close

together due to the pressure of the investing cuticle. The labrum has become conspicuous. The mandibular palp has grown longer but still lies bent inside the integument. The maxilla is much longer than the maxillule and shows division into lobes at the tip. The first thoracic limb is shorter than the rest which are all of the same size and show a pointed spine at the tip. All the limbs are directed backwards. The first five abdominal appendages appear as small finger-shaped projections in linear series with the thoracic limbs. Of these the first is the smallest and the fifth the largest. The uropods have elongated considerably and are found to press their way backwards below the posterior end of the embryo which has now shrunk to form the telson. Near the base of the endopodite of the uropod the statocyst sac has formed as a deep thick-walled invagination from the dorsal side. The eyes have become rounded off, but still remain close to the head due to the presence of the larval cuticle. A faint pigmentation is visible at their tips. The mouth now lies between the antennary and the mandibular segments and the stomodæum is getting elaborated into the stomach. The proctodæum has become deeper and due to the backward pushing of the place of origin of the uropods it comes to lie along the long axis of the embryo with the anus facing backwards. The carapace is being formed as a reduplication of the dorso-lateral ectoderm and its inner walls consisting of very large cells are found to be much thicker than the outer (Fig 38). The median dorsal organ has become very active and shows a large number of darkly staining bodies in its cytoplasm. It measures about 50 microns in breadth and half so in depth. The yolk septum has grown further back and along its inner edge the preantennular mesoderm of either side sends up an upper strand which reaches as far as the dorsal organ and gets attached to it. The two upper strands do not lie close together, but leave a hæmocœlic space between, which later forms the lumen of the anterior aorta. The paired cœlomic cavities of the preantennular mesoderm expand into the upper strand and reach as far as the dorsal organ. The basal portions of the preantennular mesoderm have expanded and spread like a collar round the stomodæum. In addition to the original pair of cœlomic cavities, which have become very much compressed, another pair are just making their appearance at the hinder regions of the mesoderm mass. In the end sac rudiment of the antennal gland a cœlomic space has differentiated and the outer end of the long duct mesoderm has come nearer the ectodermal layer of the antennal base. The ectoderm lying against the tip of this duct primordium invaginates and gets connected with it (Fig 35). But no cavity is as yet visible in the duct rudiment and even the walls of the ectodermal invagination lie collapsed and do not leave any space between. The edges of each liver rudiment curl inwards more and more and at the posterior

end they have already met to form a tube which is blind behind. The mesoderm of all the trunk segments has now divided into dorsal and ventral masses, the former having moved upwards along the sides of the embryo between the yolk mass and the ectoderm. The dorsal and the ventral longitudinal muscles are well developed. In all the trunk segments, except the vestigial seventh abdominal segment a coelomic cavity is differentiated in each dorsal mesoderm mass which has united intersegmentally and become continuous. I have not been able to trace the fate of the coelom in the second maxillary segment. Probably it fuses with the coelom of the first thoracic segment as in *Hemimysis* (Manton, 1928), for the cavities in the latter are found to be much larger than those in the succeeding ones. At the dorsal side of the embryo the dorsal mesoderm has become attached to the ectoderm a short distance on either side of the mid-dorsal line and due to the shrinking of the yolk-sac three hæmocœlic spaces have been formed. These are, the median heart cavity and the two dorso-lateral pericardial spaces. The mesoderm has also united across the floor of the median cavity immediately above the yolk-sac to form the ventral wall of the heart. From this a thin tongue of mesoderm grows down on either side and invests the yolk-sac. The genital rudiments have now reached the dorsal side and have fused to form a single mass, lying below the pericardial floor in the first thoracic segment (Fig. 40). The transformation of the layer of yolk cells into the midgut epithelium is progressing fast from the posterior endoderm plate forwards.

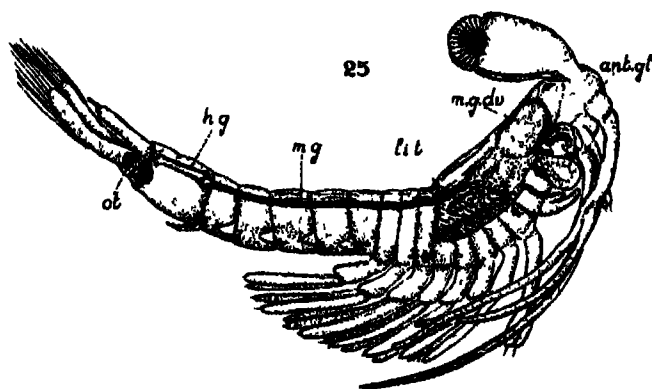


FIG 25

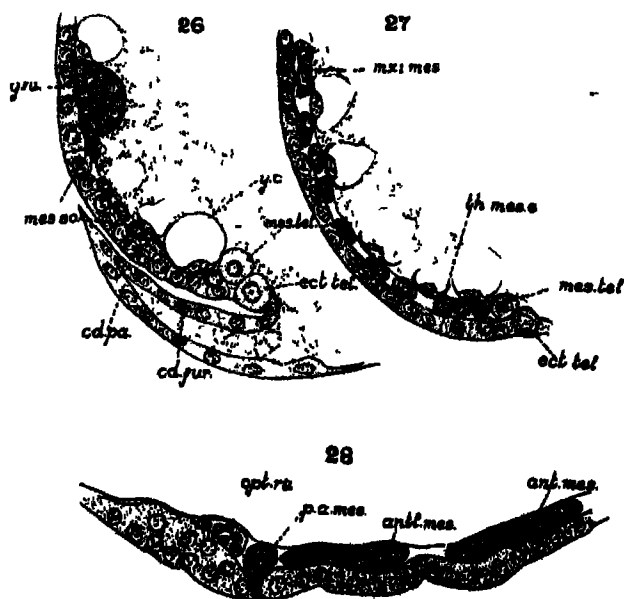
Stage VII —

FIG. 25

Length (straight from head to telson)	..	1330 microns
Length of antennule	..	700 "
Length of antenna	..	840 "
Length of antennal palp	262	"

This stage occurs soon after the first larval ecdysis and shortly before the liberation of the larva from the brood pouch. The body is elongated and the appendages have become free and movable. The preoral region has been rotated forwards. The eyes are darkly pigmented and stand raised from the body on their stalks which are bent backwards. The antennule is long and consists of a stout basal stalk and two branches, of which, one is shorter than the other. The branches as well as the stalk are many-jointed and bear fine setæ. Both the antennule and the antenna lie bent backwards along the sides of the body and when the animal is lying with the dorsal side up the body actually rests on these as if on springs. The antenna is larger than the antennule and bears a thick palp at its base. The filament is segmented and bears setæ. The mandibles are fully formed and appear heart-shaped in profile. The mandibular palp is clearly seen projecting outwards with a small tuft of setæ at the tip. The labrum is large and rounded and measures one and a half times the mandible in lateral dimension. Both the labrum and the mandible are moved frequently. The maxillule is short and stumpy. The maxilla is much better developed and bears two lobes, both digitorial. The hinder one is longer and bends forwards below the one in front. All the thoracic legs have become biramous. The first thoracic leg is much shorter than the rest, especially the endopodite and is not so much bent backwards as the succeeding appendages. Consequently it stands a little stiff like the mandibular palp. In the remaining thoracic legs the exopodites and the endopodites are found to be almost equal in length, though the former is flat and pointed and the latter blunt and finger-shaped. The setæ are found only at the tips and no joints are visible as yet. The exopodites are frequently moved about. The carapace is well formed and, at its posterior end, stands away from the body on either side. The raised portions of the carapace show regularly arranged blood spaces inside and their inner wall remains of the same thickness as before. The anterior thoracic segments have become much crowded and the abdomen shows six clear cut segments, the last one being twice as long as any other. The uropods and the telson are well developed, though not showing the final shape. The former appear as blunt finger-shaped structures bearing long setæ at the tip. The statocyst sacs have become deeper but are empty. All the other abdominal appendages are small and leaf-like with the exception of the fifth pair and all of them lie close to the body. The living embryo is transparent allowing many of the internal details to be studied under the microscope. A small quantity of yolk still remains to be absorbed in the midgut diverticulum and in the space in front of the stomodæum some yolk degenerates. The liver consists of three pairs of thick-walled tubes opening into the midgut very near its

junction with the foregut, in two clusters on either side. It will be remembered that originally the liver consisted only of a single tube on either side. The present condition is attained by the longitudinal splitting of this pair of tubes. The division takes place along the horizontal plane from behind forwards. Of the three pairs in the present stage the lowermost are the largest. The adult possesses four pairs of liver tubes and so another pair are added later on to the existing ones. These are formed not by division as before, but as two pouches from the place of union of the three tubes described above. By the time the first pair of liver tubes are formed the transformation of the yolk cells into the midgut epithelium in continuation of the posterior endoderm plate would have advanced far into the front. The pair



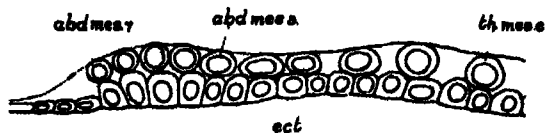
FIGS 26-28

of liver tubes now begin to pulsate vigorously and pump the yolk into the hinder regions of the midgut which is very much shrunken and closed behind. A continuous peristalsis results from this and the yolk is kept in motion throughout the midgut. The proctodæum is closely apposed to the midgut and it fits on to the rounded end of the latter like a double-walled cup in the sixth abdominal segment. The proctodæal part of the double layered septum that is thus formed is thinner and is the first to dwindle off, though communication between the midgut and the hindgut is not established until after the liberation of the larva from the brood pouch. At the anterior end on the other hand the stomodæum has elaborated itself into the stomach

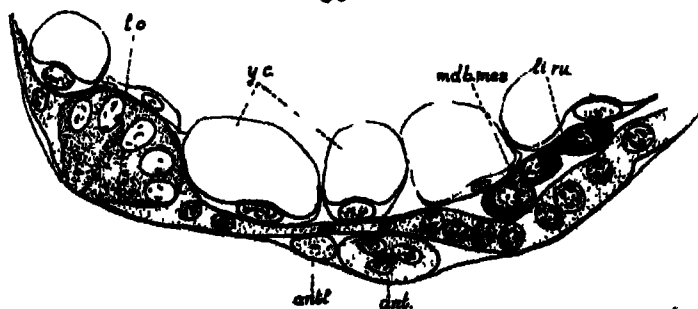
and is already in communication with the midgut even though some yolk still remains to be absorbed in the dorsal diverticulum of the latter. The posterior end of the stomach projects into the midgut like the tail of a funnel (Fig 41). In the living larva the stomach is found to expand and contract rhythmically.

The median dorsal organ has now disappeared completely and the anterior aorta that is formed between the upper strands is brought in touch with the front end of the heart, while the strands themselves have transformed into muscles in association with the stomach. The first formed preantennular coelomic cavities have disappeared and a secondary pair have developed on either side of the stomach and fused together below it. The formation of the antennal gland is almost complete at this stage, the lumen having developed in the duct mesoderm and established communication with the exit tube at one end, while the other extremity lies in close contact with the end sac. The inner end of the ectodermal exit tube does not form a chimney of cells projecting into the cavity of the duct as was observed by Manton (1928) in *Hemimysis*. The end sac is small and thick-walled and lies in a

29



30



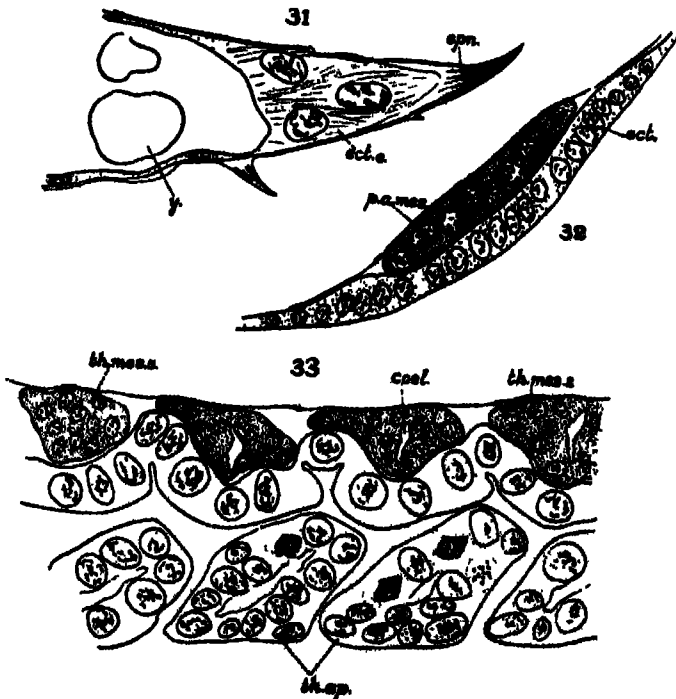
FIGS. 29-30

fold of the ectoderm immediately in front of the mandible. The duct is long and U-shaped and lies bent backwards in the cephalic cavity where yolk was said to degenerate, closely apposed to the yolk mass along part of its length. Near its exit the duct is distended. The heart appears as a long

and thick-walled tube in the dorsal side of the thorax, but it does not as yet communicate with the anterior aorta, there being a plug of cells in between. The posterior aorta is very much shrunken and lies completely separated from the mid-dorsal ectoderm in free communication with the hinder end of the heart. The hepatic and sternal arteries are also formed and the afferent venous channels have been established. The coelomic cavities in the pericardial floor are clear and appear as thin-walled sacs arranged in linear series from the first thoracic segment backwards. The genital rudiment is enclosed in a sheath of mesodermal cells and the rudiments of the gonoducts are being differentiated from the pericardial floor behind it.

Post-Larval Development

Inside the brood pouch the larvæ lie with their head directed backwards and the dorsal side up. From the very earliest stage they orientate themselves in this manner. The mother ensures a constant supply of water inside the brood pouch by gently opening and closing the pouch lamellæ while swimming. Undoubtedly the spines that are very early formed on the body of the embryo are of great help to keep them together and to prevent their falling off during the ventilating operations of the mother.



Figs. 31-33

As soon as the small quantity of yolk in the midgut diverticulum, described under Stage VII, is absorbed the larva is liberated from the brood pouch. This happens invariably during night time, 96 hours after the eggs are extruded, as was described earlier in the paper. By this time the larvæ have increased in size considerably and possess long biramous appendages. Consequently they find themselves cramped up for space within the brood pouch of the mother. The increasing mobility of their limbs add to their discomfort and not infrequently the brood pouch lamellæ are pushed asunder by their pressure. Finally without much visible effort on the part of the mother, which keeps on swimming all the time, the lamellæ open out widely and the larvæ drop down one after another. Though all the organs have been formed the larvæ are still bent dorsally and are unable to swim. The appendages have not attained their final form and the otolithic vesicle is empty and so, as soon as they are liberated, the larvæ sink to the bottom. Here they undergo a second ecdysis after which the body becomes straight and the larvæ are able to swim. In about ten to twenty minutes after liberation the tiny young ones could be seen swimming about slowly far below the surface. The eyes, the antennules and the antennæ have now swung forwards and all the appendages and the telson show the final features with characteristic spines and setæ. In the uropods the otoliths have made their appearance. Though neither the brood pouch nor the penis is developed the sex of the young one could be determined by the dimorphism exhibited by the antennules and the abdominal appendages. Internally the septum separating the midgut and the hindgut has disappeared. The gastric armature is fully developed and the last pair of liver tubes are visible in rudimentary form. Large vacuoles have already appeared in the liver cells. The circulatory system is in complete working order, but the reproductive organs have not developed fully. The cells of the gonad show active proliferation, but a cavity is yet to appear in the gonoduct rudiment.

Table I gives in broad outline an idea of the subsequent growth of the young ones till maturity.

The youngest female with the upper ovarian tubes filled with large yolk-laden eggs visible externally measures 4 mm in length. The eggs are approaching the end of their growth period but the brood pouch of the animal is incomplete. With the next moult the pouch will be fully formed and the eggs will be extruded into it. Similarly the youngest male containing active spermatozoa measures 3.5 mm. It has not been possible to ascertain the time the animals take to grow to this size, but judging from the rapidity of the early development and the maximum dimensions reached by the adult it appears that *Mesopodopsis* attains sexual maturity very early in life.

TABLE I
Table showing Post-larval Development

No	Sex	Length	Condition of gonads	Ext repr organs	Internal organs
		mm			
1	Male	2.3	Not visible outside	Not developed	Last liver tubes short
2	Female	1.5	do	do	do
3	Female	1.6	do	do	do
4	Male	1.4	do	do	do
5	Male	1.5	do	do	do
6	Female	1.7	do	do	do
7	Female	2.2	do	do	do
8	Female	1.4	do	do	do
9	Female	2.5	do	do	do
10	Female	1.5	do	do	do
11	Male	2.5	do	do	Liver well developed
12	Male	2.6	do	do	do
13	Female	2.2	do	do	do
14	Male	2.8	do	do	do
15	Male	3.0	Testes developed	Stumps, 0.2 mm	Dark substance in ant gl
16	Male	3.2	do	do	do
17	Female	4.5	Eggs visible outside	Pouch incomplete	do
18	Male	3.5	Testes developed	Stumps, 0.3 mm.	do
19	Male	3.2	do	Small stumps	Gland duct empty
20	Female	4.0	Eggs visible outside	Pouch incomplete	do

Discussion on Development and Organogeny

The differences exhibited by *Mesopodops*s in the matter of segmentation have already been discussed. Regarding the later stages, though there is agreement in the general course of development of the form and other Mysids, yet the present investigation has brought to light a number of features which are interesting. These may now be considered.

The caudal furrow in *Mesopodops*s occurs immediately behind the ectodermal teloblasts, that is right across the blastopore. That this is so is

also proved by the position of the teloblasts in Stage II (Fig. 26), just anterior to the angle of the furrow and not on the ventral side of the papilla itself as occurs in *Hemimysis* (Manton, 1928, Pl 21, Fig 6) and *Mysis* (Bergh, 1893, Pl 29, Fig 36) The same thing also occurs in *Nebalia* (Manton, 1934) If the furrow falls above the row of teloblasts, the latter can never occupy a similar position unless they shifted forwards But this never happens On account of the more posterior position of the furrow the caudal papilla in *Mesopodopsis* is much shorter than that of *Hemimysis* and barely reaches as far as the mandibular segment

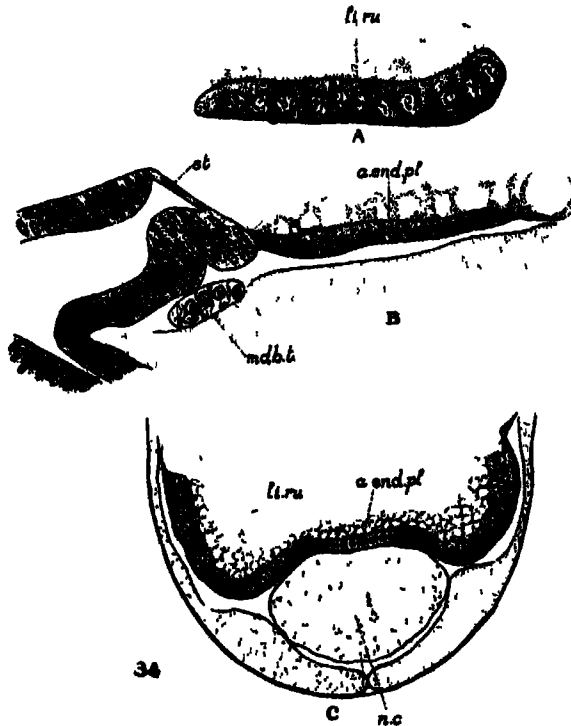


FIG 34

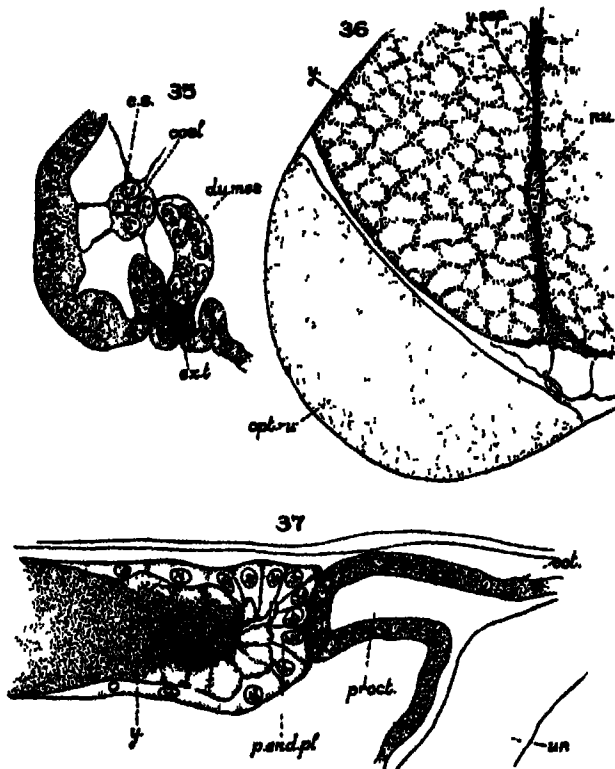
Gastrulation by immigration of cells from the area lying behind the teloblasts ceases immediately the caudal furrow deepens when only the maxillary segment is laid out In this early completion of gastrulation and the consequent closure of the blastopore *Mesopodopsis* resembles *Leander* (Sollaud, 1923), *Astacus* (Reichenbach, 1886) and *Nebalia* (Manton, 1934) and disagrees with *Hemimysis* where the formation of endoderm is a much prolonged process and the closure of the blastopore happens only when all the thoracic and three abdominal segments have been formed (Manton, 1928).

An embryonic seventh abdominal segment which later fuses with the sixth occurs in *Mesopodopsis* and it is formed in the same manner as in *Hemimysis* by the direct transformation of the teloblasts after they have given rise to the sixth abdominal segment. A paired caudal furca has been shown to occur in the early stages by almost all previous workers on Mysid embryology and hence its absence in *Mesopodopsis* is remarkable. In spite of very careful examination of both living and fixed material as well as serial sections I was not able to see anything resembling the structures figured by Nusbaum (1887), Bergh (1893) and Manton (1928). The tip of the caudal papilla is provided only with the apical spines described under Stage II, which are purely cuticular structures like the spines in the other regions of the body. Manton (1934) is inclined to think that the deciduous caudal furca of *Hemimysis* may be considered homologous with the large jointed furca of *Nebalia*, because of the topographical similarity of the two structures. But in view of the fact that in *Mesopodopsis* the furca is not developed at all this becomes very doubtful.

It has been shown that in *Mesopodopsis* the third pair of naupliar appendages are suppressed during early development. This seems to be exceptional. They have been shown to be very clearly formed as ectodermal tubes projecting outside the larval integument during the early development of *Mysis chameleo* (Nusbaum, 1887), *Mysis neglecta* and *Mysis flexuosa* (Bergh, 1893) and *Hemimysis lamornei* (Manton, 1928). It is probable that Boutchinsky (1890) and Wagner (1902) have also observed them in *Parapodopsis cornuta* and *Neomysis vulgaris* respectively, since none of the later workers has mentioned anything about the matter. In *Mesopodopsis* the body of the mandible gets raised from the general surface late in development and the mandibular palp is formed entirely within the first larval integument. But in spite of this external suppression of the third pair of naupliar appendages, the development of the antennular, antennal, and mandibular segments and the formation of the head mesoderm bands are in close agreement with the Peracarida as typified by *Hemimysis*.

Teloblastic formation of the ectoderm and mesoderm evenly from before backwards takes place only behind the mandibular segment. Nothing need be said concerning the formation of the endoderm in *Mesopodopsis*. It is very similar to that found in *Hemimysis*, with this difference that the immigration of yolk cells from the surface ceases very early. Regarding the liver Manton (1928) showed for the first time that in *Hemimysis* it originates from the head mesoderm in the mandibular segment. Quite recently Needham (1937) has disputed this and contends that in the development of *Neomysis vulgaris* it originates from the layer of yolk cells. The technique

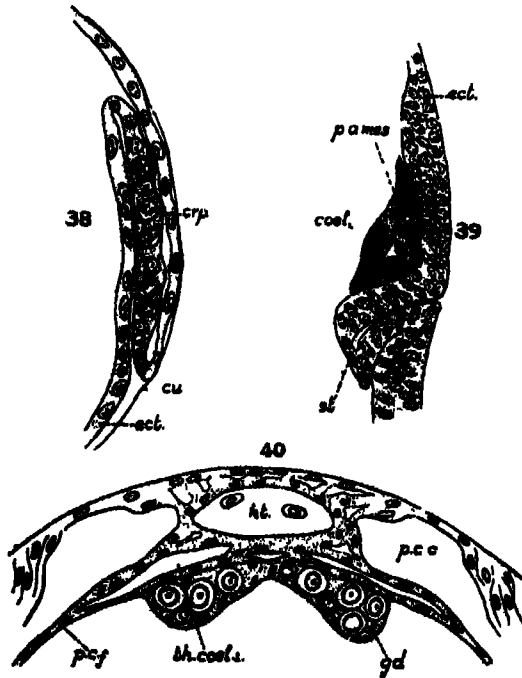
used by Needham is not the best suited for critical work in Crustacean embryology and his arguments and figures are far from convincing More



FIGS. 35-37

than everything the author himself admits that it has not been possible to demonstrate the ultimate origin of the liver and that the problem may well repay further investigation His evidence is therefore purely topographical and circumstantial But even these fail in the face of the observations on *Mesopodopsis* recorded above Here, long before the appearance of the stomodæum on the scene, the paired liver primordia are differentiated from, and lie in close proximity to, the mandibular mesoderm with the layer of yolk cells passing undisturbed over them. The condensation of the anterior endoderm plate takes place only later and there is no similarity, histological or cytological, between this and the liver rudiments. Unlike the liver, the posterior and the anterior endoderm plates never appear as cell masses separated from the yolk sac, but possess from the very beginning the characteristics of true condensations at its periphery Thus Manton's

important contribution is supported by the present investigation on *Mesopodopsis* and it seems certain that better technique and more detailed examination will show that this process is universal in the Peracarida. There is one noteworthy difference, however, in the growth of the liver rudiments in *Mesopodopsis*. They remain paired and totally separate from each other throughout development and have not been found to unite along the median line at any time. In *Hemimysis*, on the other hand, they are paired in the beginning, then unite medially for a longer or a shorter period, and again separate laterally and curl up. The transformation of the yolk cells into the midgut epithelium starts from two localized regions, at the anterior and posterior ends, where it happens precociously as in *Neomysis* (Wagner, 1898) and *Hemimysis* (Manton, 1928), but the anterior endoderm plate lies not exactly in the same position as in those two forms. There it is formed



FIGS. 38-40

opposite the stomodaeum, but in *Mesopodopsis* it makes its appearance in the mandibular segment, between the two liver rudiments just when they are curling off and does not extend over the tip of the stomodaeum. In later development the tip of the stomodaeum projects over the anterior endoderm plate for a short distance and this condition persists in the adult as the

funnel-shaped posterior end of the stomach abutting into the beginning of the midgut

The origin, development and final fate of the preantennular, head and trunk mesoderms of *Mesopodopsis* show little that is at variance with *Hemimysis*. There is no trace of any somite developing anterior to the preantennular as was claimed by Wiesmann (1926) for *Carausius morosus*. Very recently Roonwal (1936, '37) has brought forward very strong evidence in support of Wiesmann to show that in *Locusta* a pair of coelomic sacs are developed in the head anterior to the preantennary (corresponding to the preantennular somite of the Crustacea as generally accepted) somite and in association with the labrum, which, he argues, should be considered as appendicular in nature. This contention upsets the existing conception of the segmentation of the Arthropod head, but since this additional pair of somites have not been shown in any other form outside the Insecta it seems a little premature to generalize. I have found nothing that could be of support to this view in the development of *Mesopodopsis*, but it has to be admitted that the preantennary somite of *Carausius* and *Locusta* cannot be explained away.

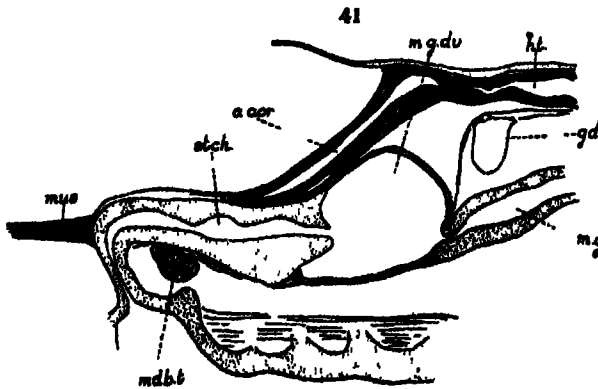


FIG 41

Regarding the head mesoderm and the development of the antennal gland some confusion has arisen lately. Manton (1928) gave a very clear account of the formation of the head mesoderm bands and proved conclusively that the antennal gland in *Hemimysis* develops from the head mesoderm in the antennal segment. But Vogt (1935) working on the development of the antennal gland in *Praunus flexuosus*, *Gastrosaccus spinifer* and *Mysis relicta* comes to conclusions which are not quite in harmony with the results of Manton to whose valuable paper he has not referred at all. He says that the cavity of the antennal evagination becomes occupied by a mass of

mesoderm originating from the ectoderm and actually figures the antennal mesoderm being proliferated from the ventral ectoderm (Figs 2 & 3, p. 484). Evidently Vogt is here following Bergh (1893) who thought that probably the naupliar mesoderm of *Mysis* is formed from the blastoderm *in situ*. But this assumption has been proved to be incorrect by Manton (1928) according to whom the naupliar mesoderm is formed of elements which have wandered forwards from the blastoporal region, a process which has been shown to happen in an identical way in the development of *Mesopodopsis* outlined in the foregoing pages. What Vogt interprets as proliferations from the ectoderm is, I think, only the head mesoderm of the antennal segment, formed by an entirely different process, lying with one edge in close proximity to the ventral ectoderm (*cf.* Manton, 1928, Text-Fig 27-a). It is unfortunate that Vogt overlooked the work of Manton and repeated the error of earlier workers which she had corrected. Regarding the antennal gland Vogt also finds that it arises from two sources and thereby homologizes the antennal gland of the Crustacea with the nephridium of the Annelida. This is at variance with the development of the organ in *Mesopodopsis* where, as in *Hemimysis*, the rudiments of the end sac and the duct though they separate very early have a common origin from the mesoderm of the antennal segment. The end sac is developed not from inwandered mesenchyme cells, as Vogt finds, but by the elaboration of the coelomic cavity that is very early differentiated in the mesodermal primordium. *Praunus*, *Gastrosaccus* and *Mysis* (Vogt, 1935) agree with *Mesopodopsis* in the absence of the chimney of cells projecting for a short time from the inner end of the ectodermal exit tube into the lumen of the duct, shown by Manton (1928) for *Hemimysis*.

Summary

- 1 *Mesopodopsis orientalis* takes 96 hours to develop from the egg to the free swimming young one
- 2 The detailed anatomy of the female reproductive system is given and the presence of a lower tube for the ovary in which the ova undergo part of their initial growth is shown.
- 3 The fully grown egg is rendered telolecithal due to the localized deposition of yolk
- 4 The process of copulation is described
- 5 The mother moults before the extrusion of the egg, which takes place at night
- 6 The lower portion of the oviduct invests the egg with a chorion
- 7 The first polar division of the egg starts while it is inside the ovary and is completed after extrusion into the brood pouch.

- 8 The polar spindle is of the anastral type
- 9 The number of chromosomes is $2N = 44$
- 10 The first polar body is very small and dwindles off soon after it is extruded
- 11 It is doubted whether the second polar body is extruded
- 12 Syngamy takes place at the centre of the egg
- 13 A fertilization membrane or vitelline membrane is formed
- 14 The zygote nucleus is situated at the centre of the egg in a stellate mass of protoplasm and the fertilized egg is typically centrolecithal
- 15 No cleavage furrows are formed
- 16 The blastomeres arrange themselves in the manner of a vesicle deep down in the yolk and rise to the surface in a body
- 17 The blastodisk is formed on the postero-ventral side of the egg
- 18 In the superficial differentiation of the germ elements the mesoderm is formed in front of the endoderm
- 19 Gastrulation takes place by immigration of cells from a blastoporal area, lying behind a row of 14 to 16 ectodermal teloblasts and eight mesodermal teloblasts below it
- 20 A genital rudiment is differentiated externally
- 21 The mesoderm of the nauphar segments is formed by cells which sink in at the blastoporal area and wander forwards
22. The post-nauphar ectoderm and mesoderm are formed evenly from before backwards by the division of the teloblasts
- 23 The caudal furrow falls across the blastopore and gastrulation ceases immediately the maxillular somite is formed
- 24 The third pair of nauphar appendages are not developed during early development
- 25 Hatching takes place by the expansion of the yolk probably due to the absorption of water
- 26 No caudal furca is formed
- 27 A preantennular somite with paired coelomic cavities is developed
- 28 The liver is formed from the head mesoderm in the mandibular segment.
29. The anterior endoderm plate lies in the mandibular segment between the liver rudiments and does not stretch over the tip of the stomodæum
- 30 An embryonic seventh abdominal segment is formed and later fuses with the sixth

31 No coelomic cavities are developed in the head mesoderm, except in the antennal gland, the development of which is also described

32 The development of the post-naupliar mesoderm is traced in broad outline Paired coelomic cavities are developed in all the post-naupliar segments excepting the maxillary and the vestigial seventh abdominal segments

33 The liberation of the larva from the brood pouch is described and a brief account of post-larval development is given

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EXPLANATION OF FIGURES

(Figs 1-41 have been reduced to half the original size)

FIG 1.—The ovary viewed from the ventral side × 80

FIG 2.—T.S of the ovary showing the opening between the upper and the lower tubes Yolk is beginning to be secreted in the eggs that have entered the upper tube. × 280.

FIG 3.—An egg from the lower tube of the ovary having no yolk at all. × 1,850.

FIG 4.—A fully grown egg from the upper tube just before maturation sets in. × 258.

FIG 5.—Copulation.

FIG 6.—Section of an egg in the upper tube showing the first polar division. × 490.

FIG 7.—The first polar spindle in metaphase × 1,850.

- FIG. 8.—The equatorial plate of the polar spindle showing 122 chromosomes $\times 2,700$.
- FIG. 9.—The first polar body $\times 1,350$
- FIG. 10.—A newly extruded egg cut *in situ* showing the chorion, the vitelline membrane and the anaphase spindle of the second polar division $\times 200$
- FIG. 11.—The same spindle magnified $\times 2700$
- FIG. 12.—The male and the female pronuclei about to unite together in the centre of the egg $\times 1350$.
- FIG. 13.—The zygote nucleus. $\times 1,350$
- FIG. 14.—Section of the egg from the brood pouch showing the first cleavage $\times 280$
- FIG. 15.—The first cleavage spindle $\times 1,350$
- FIG. 16.—Camera lucida drawing from a whole mount of the blastoderm showing the superficial differentiation and the spatial relations of the germ elements. $\times 450$
- FIG. 17.—Parasagittal section of the egg showing the process of gastrulation $\times 450$
- FIG. 18.—Sagittal section showing the beginning of the caudal furrow $\times 450$
- FIG. 19.—Stage I $\times 150$
- FIG. 20.—Stage II $\times 150$
- FIG. 21.—Stage III. $\times 135$
- FIG. 22.—Stage IV. $\times 144$
- FIG. 23.—Stage V $\times 144$
- FIG. 24.—Stage VI. $\times 125$.
- FIG. 25.—Stage VII $\times 120$
- FIG. 26.—Sagittal section of the embryo at Stage II showing the position of the teloblasts with respect to the caudal furrow $\times 450$
- FIG. 27.—Parasagittal section of the same egg as shown in Fig. 26, showing the series of mesodermal somites $\times 450$
- FIG. 28.—Parasagittal section of the early embryo showing the preantennular mesoderm slipping in. $\times 630$.
- FIG. 29.—Camera lucida drawing from living embryo showing the seventh abdominal mesodermal somite $\times 620$
- FIG. 30.—T.S. through the mandibular region of the embryo at Stage II (Only one side is shown.) The lateral organ, the mandibular mesoderm and the layer of yolk cells are clearly seen $\times 900$
- FIG. 31.—Sagittal section of the posterior end of the embryo at Stage IV $\times 1,350$.
- FIG. 32.—T.S. of the head region showing the preantennular mesoderm of one side spreading backwards. $\times 900$
- FIG. 33.—Parasagittal section of the thoracic region of the embryo at Stage V, showing the segmental dorsal mesoderm masses of one side with clear cut coelomic cavities. $\times 1,350$.
- FIG. 34.—The anterior endoderm plate and the liver rudiments A and B, in longitudinal section and under the same magnification. $\times 900$. C, Transverse section $\times 450$
- FIG. 35.—T.S. of the embryo through the base of the antenna, showing the rudiment of the antennal gland. $\times 900$.
- FIG. 36.—T.S. of the head region showing the yolk septum $\times 1,350$.
- FIG. 37.—Sagittal section of the posterior end of the embryo showing the posterior endoderm plate and the proctodæum. $\times 900$.

- FIG. 38 —T.S. of the embryo showing the carapace being formed as a reduplication of the dorso-lateral ectoderm $\times 900$
- FIG. 39 —Sagittal section of the anterior end of the embryo. The preantennular mesoderm lies in close apposition to the anterior face of the stomodæum and a coelomic cavity has appeared in it. $\times 630$
- FIG. 40 —T.S. of the embryo at Stage VII, showing the coelomic cavity in the pericardial floor, the gonad and the formation of the heart $\times 900$
- FIG. 41 —Median sagittal section of a fully grown embryo, showing the relation between the stomach and the midgut, and the heart and the anterior aorta $\times 450$
- FIG. 42.—Photomicrograph of the fully grown egg showing the peripheral position of the nucleus
- FIG. 43 —Photomicrograph of the first polar spindle. The egg is within the upper tube of the ovary
- FIG. 44 —Photomicrograph of a transverse section of the female fixed while extruding the egg. One egg is just falling into the brood pouch and is already covered with the chorion
- FIG. 45.—Photomicrograph of the first polar body lying close to the egg
- FIG. 46 —Photomicrograph of an egg lying in the brood pouch and showing the chorion as well as the vitelline membrane.
- FIG. 47 —Photomicrograph of the second polar spindle. Same as shown in Figs 10 and 11. The fertilization membrane and the sperms are clearly seen.
- FIG. 48 —Photomicrograph of the section showing the two pronuclei about to unite together. Same as Fig 12.
- FIG. 49 —Photomicrograph of the first cleavage spindle. Same as Fig. 15
- FIG. 50 —Photomicrograph of a cleavage nucleus of the four-celled stage
- FIG. 51 —Photomicrograph of the blastodermic vesicle rising to the surface from the depth of the yolk

LIST OF ABBREVIATIONS

<i>a. aor.</i>	. Anterior aorta	<i>du. mes</i>	. Mesodermal rudiment of the duct of the antennal gland.
<i>abd. ap</i>	. Abdominal appendages	<i>d. o.</i>	. Dorsal organ
<i>abd. mes</i>	. Abdominal mesoderm	<i>e.</i>	. Eye
<i>a. end. pl.</i>	. Anterior endoderm plate	<i>ect.</i>	. Ectoderm
<i>ant.</i>	. Antenna	<i>ect. c.</i>	. Ectodermal cells
<i>ant. gl.</i>	. Antennal gland	<i>ect. tel.</i>	. Ectodermal teloblasts.
<i>antil.</i>	. Antennule	<i>end. c.</i>	. Endodermal cells
<i>antil. mes</i>	. Antennular mesoderm.	<i>e. s.</i>	. End sac of the antennal gland.
<i>ant. mes</i>	. Antennal mesoderm	<i>ex. t.</i>	. Exit tube of the antennal gland
<i>ast.</i>	. Aster.	<i>f. r.</i>	. Formative region of the ovary
<i>bl.</i>	. Blastema	<i>gd.</i>	. Gonad
<i>blast.</i>	. Blastomeres	<i>g. ru.</i>	. Genital rudiment.
<i>c. b.</i>	. Central bodies.	<i>g. v.</i>	. Germinal vesicle
<i>cd. fur.</i>	. Caudal furrow.	<i>h. g.</i>	. Head gut.
<i>cd. pa.</i>	. Caudal papilla	<i>h. h.</i>	. Heller Hof
<i>ch.</i>	. Chorion.	<i>h. mes.</i>	. Head mesoderm.
<i>cl. spl.</i>	. Cleavage spindle		
<i>coel.</i>	. Coelom.		
<i>crp.</i>	. Carapace		
<i>cu.</i>	. Cuticle		



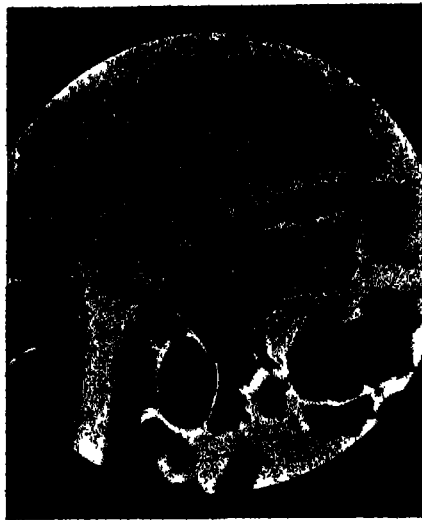
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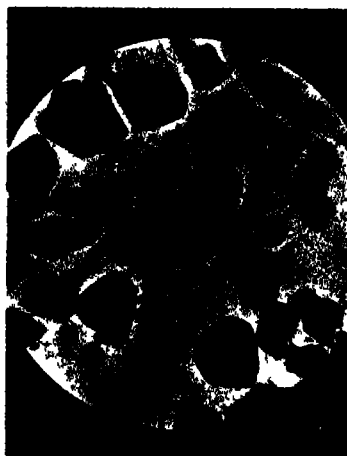
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51

<i>h.</i>	.. Heart.	<i>ov 2 w</i>	. Wall of the upper tube of the ovary.
<i>h.ru.</i>	.. Liver rudiment.		
<i>l.o.</i>	.. Lateral organ.	<i>p a mes</i>	Preantennular mesoderm
<i>l.t.</i>	.. Liver tubes.	<i>p b</i>	. Polar body.
<i>mdb.</i>	.. Mandible.	<i>p c c</i>	.. Pericardial cavity.
<i>mdb.mes.</i>	.. Mandibular mesoderm	<i>p c f</i>	Pericardial floor
<i>mdb.p.</i>	.. Mandibular palp.	<i>p end pl</i>	. Posterior endodermal plate.
<i>mdb.t.</i>	. Mandibular tendon.	<i>p nu</i>	. Pronuclei
<i>mes.so.</i>	.. Mesodermal somite	<i>pr</i>	.. Protoplasm
<i>mes.tel</i>	. Mesodermal teloblasts	<i>proct</i>	. Proctodaeum.
<i>m.g.</i>	.. Midgut	<i>s</i>	. Sperms
<i>m.g dv.</i>	. Midgut diverticulum	<i>sph</i>	. Sphere of the central apparatus
<i>mus.</i>	.. Muscle.		
<i>mx 1</i>	. First maxilla (maxillule)	<i>spl</i>	Spindle.
<i>mx 1.mes</i>	Maxillary mesoderm	<i>spn</i>	Spine
<i>mx 2.</i>	.. Second maxilla.	<i>stch</i>	Stomach
<i>n c</i>	. Nerve cord	<i>t</i>	Telson.
<i>nu</i>	Nucleus	<i>th ap</i>	. Thoracic appendage
<i>nul.</i>	. Nucleolus	<i>th coel</i>	. Thoracic coelom.
<i>opt ru</i>	. Optic rudiment	<i>th mes</i>	. Thoracic mesoderm
<i>ot.</i>	.. Otolith.	<i>ur.</i>	. Uropod
<i>ov 1.</i>	.. Lower tube of the ovary	<i>v m</i>	.. Vitelline membrane
<i>ov 2.</i>	.. Upper tube of the ovary.	<i>y</i>	. Yolk.
<i>ov.d.</i>	.. Oviduct.	<i>y c</i>	. Yolk cells
<i>ov o.</i>	.. Opening between the two tubes of the ovary	<i>y sep</i>	. Yolk septum
<i>ov 1. w</i>	.. Wall of the lower tube of the ovary	<i>z m</i>	. The zone of migration of the germ cells in the middle piece of the ovary

ON A NEW SPECIES OF *URAEOTYPHLUS* FROM SOUTH INDIA

By B R SESHACHAR

(Department of Zoology, University of Mysore, Central College, Bangalore)

(With one plate and two text-figures)

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(Communicated by Prof A Subba Rau)

IN the course of my studies on the spermatogenesis of the local forms of Apodan Batrachians which I have been investigating for some years, I have had opportunities of obtaining a fairly large collection of material. I have been able to record a few interesting phases in the phenomenon of spermatogenesis in *Ichthyophis glutinosus* Linn and with the object of extending my observations to all the species available in India, I procured fresh material from Kottayam, a small town in Travancore State. Malabar forests, it is well known, with their spurs projecting into Mysore, Coorg and as far as North Canara, form the most favourable situation for harbouring a varied and rich Amphibian fauna. It is not therefore surprising that practically all the known Indian members of Apoda are reported from this region, except the solitary instance of *Herpele Fulleri* Alc which has been described from Assam. Of these, *Ichthyophis* enjoys a wide distribution while species belonging to the genera *Uraeotyphlus* and *Gegenophis* are, so far as is known at present, confined to the western belt of Malabar and Wynaad. We have now five species definitely assigned to the genus *Uraeotyphlus* and of these, *U seraphim* Dum occurs in Africa and the other four, *U oxyurus* Dum. and Bibr, *U malabaricus* Bedd, *U menoni* Annan and *U narayani* n sp seem apparently restricted to a comparatively small area. *Gegenophis carnosus* Bedd is known only from Wynaad and Travancore and has not been taken from other parts of India. The concentration of Apodan population in the western ghats must give rise to many interesting problems in the study of the distribution of these amphibians and when our knowledge of all the ecological factors involved in the reaction of these animals to their environment is completed, we may be able to account for the abundance of Apodan life within a narrow zone. The members of the three genera, *Ichthyophis*, *Uraeotyphlus* and *Gegenophis*, occur in South India in identical physical conditions and naturally *Uraeotyphlus* and *Gegenophis* may be expected to be co-extensive with *Ichthyophis*. In this connection it may be noticed that

another interesting Apodan genus *Herpele* whose congeners occur in tropical America and West Africa has been reported from the northeastern corner of India. The discontinuous distribution of *Herpele* is accounted for by Alcock (1904) by invoking the theory of a Tethyan Sea which, as he has pointed out, explains the remarkable coincidence of the occurrence of a large number of sub-littoral genera of hermit crabs wherever *Herpele* and other Apodan genera are found. He explains the alternate theory of a land-connection well known by the name "Lemuria" as a probable explanation of the zoogeographical problems concerning *Herpele* and *Uraeotyphlus*. It is probable that when all the twenty genera including *Idiocranium* from Africa described recently by Parker (1936) into which the order Apoda Batrachia is divided should be investigated, they may be found to be erected "on comparatively slight grounds, and several of these genera are probably unnatural, the distinctive characters having undoubtedly been developed independently in various countries" (Gadow, 1923, p. 89). The problems of distribution can be understood when geological evidence of a sufficiently convincing nature supported by zoological testimony and by ecological studies is accumulated and until then, our interpretation must to a large extent be tentative.

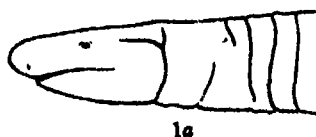
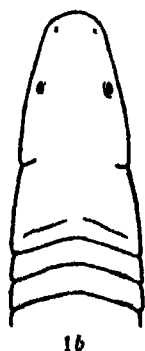
While examining the Kottayam material I found that the specimens differed in material respects from the published descriptions of *Uraeotyphlus oxyurus*, *U. malabaricus* and *U. menoni*. In order to clarify the position a few specimens from Kottayam were forwarded to the Director, Zoological Survey of India, for the purpose of comparison with the collection in the Indian Museum and Dr. Baini Prashad reported that they differed from the material in his possession. However, as *U. malabaricus* was not contained in the material at his disposal, he advised the despatch of some specimens to Dr. Malcolm Smith with a request that they may be examined more fully with reference to the collection of Apoda in the British Museum. The result of these two examinations was that the Kottayam specimens differed from the known forms and that they probably represented an undescribed species. I take this opportunity of thanking Dr. Baini Prashad and Dr. Malcolm Smith for their numerous acts of kindness and courtesy. In this connection I must gratefully record the encouragement given to me by Dr. Baini Prashad who has generously placed the type and co-type of *Uraeotyphlus menoni* for my study and my warmest thanks are due to him for this singular act of friendship. Throughout my work I have received numerous helpful suggestions from Prof. A. Subba Rao whom I have great pleasure in thanking.

I have great pleasure in associating with this new species of *Uraeotyphlus* the name of Prof. C. R. Narayan Rao, till very recently Professor of Zoology

of this University whose work on Batrachia has been a source of great inspiration to me and to whom I am indebted for numerous kindnesses during my association of over twelve years

Uraeotyphlus narayani n sp

<i>Body</i>	Cylindrical, smooth, greatest thickness which is in the middle $1/22$ to $1/17$ of body length 150-180 folds dorsally
<i>Head</i>	Elongated, prominent, rounded anteriorly. Slightly flattened dorsoventrally Snout longer than distance between the eyes, far overhangs the mouth Length of head $1/23$ to $1/20$ of body length
<i>Eyes</i>	Distinct Diameter of eye about $1/4$ of interorbital width.
<i>Tentacle</i>	Below or slightly in front of the nostril Retracted in spirit specimens
<i>Teeth</i>	Moderate Both rows in the mandible well developed, Teeth in the anterior row larger than those in the posterior row
<i>Tail</i>	Short, pointed, with 3-4 complete rings Vent in the form of a longitudinal ventral slit interrupting 4-5 rings.
<i>Colouration</i>	Dorsal surface steel grey Ventral surface pale and flesh coloured except on the throat and also posteriorly where it is dark A pale spot round the vent
<i>Occurrence</i>	The specimens were collected at Kannam, 16 miles from Kottayam, Travancore Elevation above sea-level 300-500 feet *



* I am indebted to Mr. C Eapen of the Biological Supplies, Kottayam, for this information.

TABLE I

Table showing the Detailed Measurements of Uræotyphlus narayani

	Type	Co-type	Range in six other specimens	Average for six specimens
	mm	mm.	mm	mm
Total length	220	225	165-200	190
Length of head	11	10	8-9	7
Length of snout	5	4-5	3.9-4.3	4
Interorbital width	4	3-7.5	3-3.6	3.2
Width of head	7-5	6-2	5.5-7	6.1
Internarial distance	3	2-8	2-2.4	2.1
Distance between eye and nostril	3-5	3	2.6-3	2.8
Distance between eye and tentacle	3-5	3	2.8-3.2	3
Length of tail	1	3-5	3-3.8	3.1
Number of body folds	175	160	150-177	158
Number of tail folds	3	4	3-4	3
Number of folds covered by vent	5	4	4-5	4

Uræotyphlus menonii Ann

I have recounted the number of folds in the two specimens of this species kindly loaned to me by the Indian Museum and find 197 folds on the body of the type (No 16707) and 195 on the body of the co-type (No 16695). These numbers differ from the statement of Annandale that there are "about 170 rings on the body". Further he states that the tail of *U. menonii* is "short, pointed bearing 10 complete rings". After careful examination I find that in the type there are 3 rings and in the co-type there are 4 rings on the tail.

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EXPLANATION OF TEXT-FIGS.

- FIG 1 a, 1 b, 1 c —Side, dorsal and ventral views respectively of the head of *Uraeotyphlus narayani* × 2
- FIG 2 —Ventral view of the tail of *U narayani* × 2

EXPLANATION OF PLATE

- FIG. 1 —*Uraeotyphlus narayani*, entire animal, natural size
- FIG 2 a, 2 b, 2 c —Side, dorsal and ventral views respectively of the head of *Uraeotyphlus narayani* × 3
- FIG 3 —Ventral view of the tail of *U narayani* ×

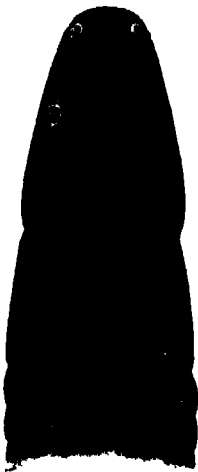


FIG 2b



FIG 2a

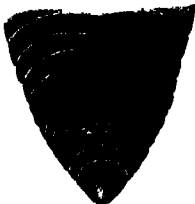


FIG 3

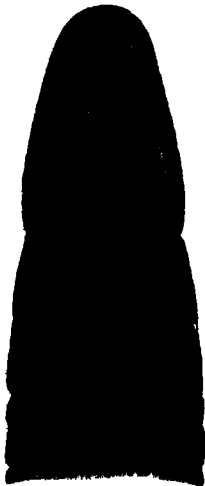


FIG 2c



FIG 1

Errata

Page 229 . First sentence, fourth word—

For "no" read "some**" and the corresponding footnote as follows :

** Bl-was, K P (1930) "Contribution to Our Knowledge of the Fresh Water
Algae of Manipur, Assam," *Journ Bomb Nat. Hist
Soc* , March 1.

————— (1934) "Observations on algal collections from Khasia and
Jaintia Hills, Assam, India," *Hedwigia*, LXXIV.

Page 231 . Fig 1 Magnification—For "370" read "440"

ON A COLLECTION OF ALGÆ FROM ASSAM*

BY P R PARUKUTTY, B Sc

Received February 28, 1939

[Communicated by Prof Y. Bhâradwâja, M Sc., Ph D (Lond), F L S.]

THERE has been no previous record of Algæ from Assam. The present paper deals with some plants collected from a few places in this province by Mr Balwant Singh, M Sc., of the College of Ayurveda in September 1936 and by Professor Y Bhâradwâja in October 1937, who kindly passed on the same to me for investigation.

Out of the twenty-six forms described here ten belong to the Chlorophyceæ and sixteen to the Myxophyceæ. One variety and five forms are new.

SYSTEMATIC ENUMERATION OF THE SPECIES OBSERVED

I CHLOROPHYCEÆ

1 *Ulotrichales*

Ulotrichaceæ

Genus *Geminella* Turpin

1 *Geminella mutabilis* (Nageli) Wille Heering, in Pascher's *Süsswasser-flora Mitteleuropas*, Heft 6, 1914, p 38, Fig 46

Forma

Lat. cell, 3.7-7.4 μ , long. cell, 5-11.2 μ , sheath, 3.7-4 μ

Habitat —Attached to stones run over by a rapid torrent at Crinoline Falls, Shillong.

The form differs from the type in much smaller dimensions.

Genus *Ulothrix* Kütz.

2. *Ulothrix variabilis* Kütz Heering, *op cit*, Heft 6, 1914, p 33, Fig 27.

Lat. cell, 5-7 μ ; long. cell, 4.8-7.4 μ .

Habitat —On wet walls near Water Works, Shillong

* From the Department of Botany, Benares Hindu University.

Genus *Microspora* Thuret

3 *Microspora floccosa* (Vauch) Thuret Heering, *op. cit.*, 1914, Heft 6, p. 150, Figs 214 and 215

Diam fil, 23–26 μ , long cell, 13–19.8 μ

Habitat —In a pond, Nalbari, Kamrup

2 *Chætophorales**Chætophoræ*Genus *Draparnaldiopsis* Smith and Klyver

4 *Draparnaldiopsis indica* Bhāradwāja Bhāradwāja, "A new species of *Draparnaldiopsis* (*Draparnaldiopsis indica*, sp. nov.)," *New Phyt.*, Vol XXXII, No. 3, 1933, p. 3, Fig 1, A, B & D

Internodal cells, 19–(46 2)–74 μ long and 25–39 μ broad, nodal cells 13–18 μ long and 25–37 μ broad, cells of short laterals, 3 3–8 μ broad

Habitat —On dead leaves in stagnant ponds, along with *Oedogonium intermedium*, Nalbari, Kamrup

Genus *Stigeoclonium* Kütz

5 *Stigeoclonium subsecundum* Kütz Heering, *op. cit.*, 1914, Heft 6, p. 70, Fig 93

Cells of main axis 5–13.2 μ thick, cells of laterals 3–10 μ thick

Habitat —In a stagnant pool, Nalbari, Kamrup.

3 *Oedogoniales**Oedogoniaceæ*Genus *Oedogonium* Link

6. *Oedogonium biforme* Nordstedt Tiffany, *The Oedogoniaceæ*, 1930, Pl. XVII, Figs 161 and 162

var *tennis* var. nov. (Fig. I)

Diaceous Macrandrous—Oogonia single, sometimes two, rarely three; ellipsoidal, poriferous, pore superior. Oospore ellipsoidal, oospore-wall smooth and thin. Antheridia in rows of 4–10, sperms two, division vertical. Basal cell elongate, apical cell obtuse, suffultory cell unswollen; vegetative cell cylindrical

Lat. cell veg. fem, 12.8–18.5 μ ; long. cell. veg. fem, 37–99.9 μ ; lat. cell veg. masc, 7.5–16.5 μ ; long. cell. veg. masc, 35–100 μ ; lat. oog.,

29.6–37 μ ; long. oog., 37–64.5 μ , lat oosp, 22.2–29.6 μ , long oosp 37–51.8 μ , lat anth, 3.3–8 μ , long anth, 35–52 μ

Habitat —In ponds attached to dead vegetable matter, Nalbari, Kamrup

The alga differs from the type in much smaller dimensions, in the oospore filling the oogonium, in the antheridia being in a much shorter chain and in the occasional presence of oogonia in series of three

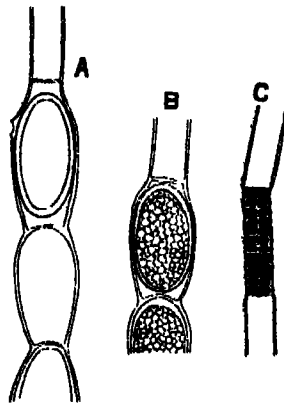


FIG 1

A—A portion of the filament showing three oögonia in series, B—a portion of the filament showing the oöspores, and C—a portion of the filament showing antheridia of *Oedogonium bifforme* var *tenuis* var nov All $\times 370$

7 *Oedogonium intermedium* Wittrock Tiffany, *op cit*, Pl XIV, Fig 134

Forma *tenuis* form nov

Monæciuous —Oögonia single, obovoid to obovoid-globose, oöspore filling the oogonium, oöspore wall smooth Antheridia 3–4, epigynous, sperms two, division horizontal Basal cell elongate, apical cell obtuse, vegetative cell cylindrical

Lat. cell veg, 13.2–16.5 μ , long cell veg, 52–59.8 μ , lat oog, 29.7–36.3 μ , long oog, 33–39.6 μ , lat oosp, 25.9–29.7 μ ; long oosp, 27–29.7 μ , lat anth, 5–6.6 μ , long anth, 13.2–15 μ

Habitat.—In ponds attached to dead vegetable matters, along with *Draparnaldopsis indica* Bhâradvâja, Nalbari, Kamrup

The form differs from the type in having slightly narrower cells

4 *Conjugales**Zygnemoides**Zygnemaceae*Genus *Spirogyra* Link

8 *Spirogyra subsalsa* Kütz Czurda in Pascher's *Süßwasser-flora Mitteleuropas*, 1932, Heft, 9, p 168, Fig 171

Lat fil, 26–429 6μ , long cell, 3–4 times as long as broad, lat. zygosp, 29–30 μ , long zygosp, 39–48 8μ , 59 5μ in exceptional cases

Habitat —In ponds along with *Anabaena sphaerica*, *Zygnema vaginatum*, *Anabaena oscillarioides*, *Anabaena fertilissima* and other algæ, Nalbari, Kamrup

9 *Spirogyra punchformis* Transeau Czurda, *op cit*, 1932, Heft 9, p 165, Fig 168

Lat fil, 19–27 μ , long. cell, 3–5 times as long as broad; lat zygosp, 33–40 μ , long zygosp, 45–57 μ

Habitat —Water-works drains, Gauhati

Genus *Zygnema* Agardh

10. *Zygnema vaginatum* Klebs Czurda, *op. cit*, 1932, Heft 9

Forma crassa form nov

Lat cell, 29–40 μ , long cell, 27–47 μ , crass zygosp, 35–40 μ

Habitat —In a pond along with *Anabaena oscillarioides*, Nalbari, Kamrup
The form differs from the type in possessing broader cells and bigger zygospores

II MYXOPHYCEÆ

1 *Hormogonales**Scytonemataceae*Genus *Tolypothrix* Kütz.

11. *Tolypothrix distorta* Kütz Gertler in Rabenhorst's *Kryptogamen-flora von Europa*, 1932, p 719, Fig 480

Lat fil, 9–16 μ ; lat. trich, 6.6–7.5 μ , long het., 13–16 μ .

Habitat —Attached to stones run over by a rapid torrent at Crinoline Falls, Shillong, along with *Geminella mutabilis*.

Nostocaceæ

Genus *Anabæna* Bory.

12 *Anabæna sphaerica* Born et Flah var *attenuata* Bhâradwâja
Bhâradwâja, "The Myxophyceæ of the United Provinces, India—I," *Proc Ind Acad Sci*, 1935, B, Vol II, No 1, p 104, Fig 5, G and H

Lat. trich, 3 3-4 μ , long cell, 3 3-4 8 μ , lat het, 5-6 μ , long het, 4 9-6 6 μ , lat spor, 10-13 2 μ , long spor, 10-16 5 μ

Habitat —In stagnant water of rice fields, Nalbari, Kamrup

13 *Anabæna oscillarioides* Bory Geitler, *op cit*, 1932, p 886, Fig 567 c

Forma

Lat cell, 4 9-6 6 μ , long cell, 5-6 8 μ , lat het, 6-10 μ , long het, 5 5-10 5 μ , lat spor, 6 6-12 μ , long spor, 15-26 5 μ

Habitat —In ponds attached to dead vegetable matter, along with *Zygnema vaginatum* and *Anabæna fertilissima*, Nalbari, Kamrup

The form agrees with the type, but differs in having smaller heterocysts and shorter spores

14 *Anabæna fertilissima* Rao Rao, "The Myxophyceæ of the United Provinces, India—III," *Proc Ind Acad Sci*, 1937, B, Vol. VI, No 6, p 364, Fig 6, A-C

Lat cell, 4 5-6 6 μ , long cell, 4 5-6 μ , lat het, 4 5-9 μ , long het, 4-6 6 μ , lat spor, 6 6-11 μ , long spor, 5 5-8 8 μ

Habitat —In ponds along with other algae, Nalbari, Kamrup.

Oscillatoriaceæ

Genus *Spirulina* Turpin

15 *Spirulina subsalsa* Oerst Geitler, *op cit*, 1932, p. 928, Fig 593 a.

Lat trich, 1 5-2 μ , lat spir, 3 5-4 μ ; spot inter due. spir, 3-5 μ

Habitat —On dead leaves in stagnant water, along with *Oscillatoria terebriformis*, Fatahil, also Barpathar and Sibsagar

16 *Spirulina maior* Kütz Geitler, *op cit*, 1932, p 930, Fig. 595.

Lat. trich., 1 5-2 μ ; lat. spir., 3 3-4 μ , spot. inter. due spir, 3-4 μ .

Habitat.—On moist soil, Fatahil, Barpathar and Sibsagar.

Genus *Oscillatoria* Vauch

17 *Oscillatoria chlorina* Kütz Frémy, *Les Myxophycees de l'Afrique equatoriale française*, 1930, p 221, Fig 183

Lat trich, 3-4 μ , long cell, 3.3-5 μ

Habitat —On moist soil, Shillong

18 *Oscillatoria terebriformis* Ag Forma, Rao Rao, "The Myxophyceæ of the United Provinces, India—II," *Proc Ind Acad Sci*, 1936, B, Vol 3, No 2 (Cf Gertler, *op cit*, 1932, p 955, Fig 607 d)

Lat trich, 4-6 μ , long cell, 3-5 μ

Habitat —On dead leaves in stagnant water along with *Spirulina subsalsa* and *Oscillatoria pseudogeminata*, Fatahil, Barpathar and Sibsagar

19 *Oscillatoria pseudogeminata* G Schmidle Gertler, *op cit*, 1932, p 966, Fig 616

Lat cell, 1.3-1.6 μ , long cell, 1.6-2.6 μ

Habitat —On dead leaves in stagnant water, along with *Spirulina subsalsa* and *Oscillatoria terebriformis*, Fatahil, Barpathar and Sibsagar

20 *Oscillatoria acuminata* Gom Gertler, *op cit*, 1932, p 950, Fig 603 d

Forma *tenuis* form nov

Lat trich, 1.6-1.8 μ , long cell, 3.3-4.8 μ .

Habitat —On soil near a hot spring, Garampani

The form differs from the type in having narrower trichomes and shorter cells

21 *Oscillatoria variabilis* Rao Rao *op cit*, 1936, p 172, Fig 3 A-D.

Lat trich, 5-6 μ , long cell, 3.3-5 μ

Habitat —On stones submerged in a running stream along with other algæ, Cherrapunji.

Genus *Phormidium* Kütz

2 *Phormidium Retzii* (Ag) Gomont Tilden, *Minnesota Algæ*, Pl. V, Fig 1-4

Lat. trich, 4.5-6 μ ; long. cell., 3.3-5.5 μ .

Habitat.—In Water-works drains, Gauhati

23 *Phormidium ambiguum* Gom Tilden, *op cit*, Pl V, Fig. 5.

Lat trich, 4–6 μ , long cell, 15–25 μ

Habitat —On swampy soil, Nalbari, Kamrup

24 *Phormidium corium* Gom Tilden, *op cit*, Pl IV, Figs 71 and 72

Lat trich, 3.7–5 μ , long cell, 3.3–7.5 μ

Habitat —On soil and stones in a shallow drain, Shillong

Genus *Lyngbya* Agardh.

25 *Lyngbya Martensiana* var *calcareo* Tild Tilden, *op cit*, Pl V, Fig 44

Lat fil, 7.4–14.2 μ , lat trich, 6.6–9 μ , long cell, 3–4 μ

Habitat —On cemented steps receiving a constant water-fall from a drain higher up, Water-works, Gauhati

Genus *Schizothrix* Kütz

26 *Schizothrix Beccarii* Gom Frémy, *op cit*, 1929, p 113, Fig 102, a and b

Lat fil, 14–24 μ , lat trich., 10–14 μ , lat cell, 2–4 μ

Habitat —Attached to stones in a shallow stream receiving dirty water near the road leading to Spreadeagle Falls, Shillong

In conclusion, I have much pleasure in expressing my great indebtedness to Professor Y Bhâradwâja, for his kind guidance and criticism throughout the course of this investigation

ECHINOIDS FROM THE BAGH BEDS—PART II

BY G W CHIPLONKER, M Sc

(Demonstrator, Department of Geology, Benares Hindu University)

Received March 9, 1939

(Communicated by Prof. L Rama Rao, M.A , F.G.S.)

SINCE the publication of the previous communication¹ on the echinoids from these strata, repeated collections of fossils were made from the various exposures of these beds. These, besides yielding two non-Indian Cenomanian species of *Hemaster*, have furnished well-preserved material enabling definite identification of some of the species which were formerly left as unidentifiable.

Description of the Species

Echinobrissus malwensis sp. nov.

Plate XXV, Fig 1

		mm	mm
Dimensions —	Length ..	23 5	20 8
	Breadth .	21 3	18 2
	Height .	10 4	9 0

Description —The test is sub-rectangular, longer than broad, with parallel sides, obtusely rounded in the front, truncated posteriorly. The summit coincides with the apical disc at about $\frac{2}{5}$ length from the front. With the advancing age the species has a tendency towards a relatively more depressed test. The upper surface slopes more steeply to the front than behind. The periproctal groove is narrow, reaches up to about $\frac{2}{5}$ length from behind and produces a shallow indentation at the posterior border. The ambulacra are lanceolate with the inter-poriferous zones slightly wider than the poriferous ones. The posterior ambulacra are slightly deflected outwardly towards the distal ends of the petaloid portion. The peristome is in a depression at about $\frac{2}{5}$ length from the front. The principal scrobiculated tubercles are crowded on the upper surface but become wider spaced on the inferior surface.

Comparison —From all the associated echinobrissi this species is very easily separated by its sub-rectangular test.

¹ *Proc Ind. Acad Sci*, (B), 6, pp. 60-71.

E. micoleti d'Orb¹ from the Urgonian of France, has its superior face generally more raised and anteriorly more precipitous. This species appears to be ancestral to the present one.

E. eddissensis Gauthier² from the Aptian of Algeria has its posterior ambulacra equal to the anterior ones and its test is ambitally more tumid.

Echinobrissus chirakhanensis sp. nov.

Plate XXV, Fig. 3

1939 *Echinobrissus* sp. indet., *Proc. Ind. Acad. Sci.*,
Ser. B, Vol. VI, No. 1, p. 63

		mm	mm
<i>Dimensions</i> —	Length	22.9	14.8
	Breadth	> 22.6	14.0
	Height	11.0	> 5.9

Description — The test is nearly as long as broad, squarely truncated behind and tapering to the front. The summit of the test, coincident with the apical disc, is situated at about $\frac{2}{5}$ length from the front. With the advancing age the apical disc moves forward from its sub-central position. The periproctal groove reaches half way between the apex and the posterior border, where it produces a feeble indentation. The upper face slopes to the front more rapidly than behind, in which direction the slope is first low until the top of anal groove is reached and then it becomes a little steeper. The ambulacra are lanceolate with interporiferous zones broader than the poriferous ones. The posterior pair of ambulacra is longer than the anterior pair, and are distally deflected outwards near the periproctal groove. The peristome is situated in a depression at about $\frac{2}{5}$ length from the front. The primary scrobicled tubercles are crowded on the upper surface, but become wider spaced on the inferior face.

Comparison — This species can be easily separated from the associated *E. malwensis* sp. nov. which has a narrower test with the sides parallel.

The associated *E. rajnathi* sp. nov. has its test, relatively longer, more tapering to the front, less truncated behind, more depressed with the sub-central summit behind the apical disc.

¹ *Pal. Fr.*, 6, p. 399, pl. 956, figs. 1-6; *Ech. Suisse*, 1, p. 40, pl. 7, figs. 4-6; *Cat. Rats*, p. 97.

² *Ech. foss. Alg.*, fasc. 3, p. 27, pl. 1, figs. 8-9, pl. 2, figs. 1-5.

E. eddisensis Gauthier⁴ from the Aptian of Algeria has its posterior ambulacral pair equal to the anterior one, ambitally it is more tumid and anteriorly less tapering

The shorter variety of *E. angustier* Gauthier⁵ from the Albian-Cenomanian of Algeria differs from the present species in being relatively longer and more rounded than truncated behind, its ambulacral pairs are equal and the upper face slopes to the front more gently.

Nucleolites similis Desor⁶ from the Cenomanian of Sarthe has its test relatively longer, its posterior border is more rounded and almost without the indentation, the anal groove extends more towards the apical disc and the posterior pair of ambulacrals is more divergent

E. expansus Clark⁷ from the Riply formation (Upper Cretaceous) is widest less posteriorly, it is more rounded behind, the anal groove extends more towards the apical disc and produces a stronger indentation at the posterior border

E. balli Fourtau⁸ from the Upper Cenomanian of Egypt, has a form very similar to that of the present species, but its summit is a little behind the apical disc, its upper face slopes behind straight

Echinobrissus rajnathi sp. nov.

Plate XXV, Fig. 2

1937 *Echinobrissus cf. angustier* Gauthier, *Proc. Ind. Acad. Sci.*, Ser. B, Vol. VI, No. 1, 63

Dimensions —	mm	mm	mm
Length	17.6	19.2	19.0
Breadth	16.2	17.2	17.6
Height	8.2	8.0	7.6

Description — The test is longer than broad, rather depressed ambitally tumid, sub-centrally highest, posteriorly more truncated than rounded, anteriorly tapering and widest at about $\frac{1}{2}$ length from behind. The upper

⁴ *Ech. foss. Alg.*, fasc. 3, p. 27, pl. 1, figs. 8-9, pl. 2, figs. 1-5.

⁵ *Ibid.*, p. 81, pl. 9, figs. 1-8.

⁶ *Ech. Sarthe*, p. 189, pl. 32, figs. 10-13; *Pal. Fr.*, 6, p. 405, pl. 958, figs. 1-4; *Synopsis*, p. 259

⁷ *John Hopkins Unv. Circ.*, 12, p. 52; *Ibid.*, 10, p. 76; *Bull. U.S.G. Surv.*, 97, p. 61, pl. 26, fig. 1; *Monogr. U.S.G. Surv.*, 56, p. 69, pl. 28, fig. 1; *Proc. Acad. Nat. Sci. Phil.*, 57, p. 6.

⁸ *Cat. Invert. Egypt*, No. 2, p. 50, pl. 4, figs. 10-11

surface is more or less evenly raised, the slope being slightly steeper behind than to the front. The apical disc is at about $2/5$ length from the front. The periproctal groove is rather narrow, reaches up to about $2/3$ the distance from the posterior border to the apical disc, and produces a feeble indentation at the posterior border. The ambulacrals are lanceolate, with interporiferous zones wider than the poriferous ones, the posterior ambulacrals are longer than the anterior ones and are slightly deflected outwards near the anal groove. The peristome is in a depression at $2/5$ length from the front. The primary tubercles are crowded on the upper surface and become wider spaced on the lower surface.

Comparison—From the associated *E. haydeni* Fourtau⁹ this species differs in having the test anteriorly more tapering and posteriorly less squarish, its summit is sub-central and the periproctal groove is narrower.

As compared to the associated *E. chirakhanensis* sp. nov. the present species has its test relatively more elongate and posteriorly less squarish with the periproctal groove longer and the posterior indentation more marked.

E. angustior Gauthier¹⁰ from the Albian and Cenomanian of Algeria has its ambulacral pairs equal, its shorter variety is more rounded behind and has its greatest width more anteriorly situated, the elongate variety has its test less tapering anteriorly, the apical disc is more eccentric to the front and the upper surface is more raised.

E. kachlini d'Orb.¹¹ from the Albian of France has its test more elongate, the posterior border more truncated, superior surface more raised and the greatest width is situated a little less posteriorly.

E. cordatus d'Orb.¹² from the Cenomanian of France is more strongly notched at the posterior border, the periproctal groove rises more towards the apical disc, the superior face is generally more raised and antero-superiorly more tumid, the interporiferous zones are much wider.

E. minimus d'Orb.¹³ from the Senonian of France and *E. roberti* d'Orb.¹⁴ from the Urgonian of France, are more elongate and less truncated behind, the upper surface is more unsymmetrically raised and the interporiferous zones are wider.

⁹ *Rec. G.S.I.*, 49, p. 44, pl. 2, fig. 1, *ibid.*, 22, p. 90; *ibid.*, p. 91.

¹⁰ *Bol. foss. Alg.*, fasc. 3, p. 81, fasc. 5, p. 145, pl. 9, figs. 1-8.

¹¹ *Pal. Fr.*, 6, p. 403, pl. 957, figs. 1-5.

¹² *Ibid.*, p. 404, pl. 957, figs. 6-9; *Cat. Rais.*, p. 96.

¹³ *Pal. Fr.*, 6, p. 414, pl. 962, figs. 1-6; *Cat. Syst.*, p. 4; *Cat. Rais.*, p. 96.

¹⁴ *Pal. Fr.*, 8, p. 397, pl. 955, figs. 1-6.

Nucleolites similis Desor¹⁵ from the Cenomanian of Sarthe has its posterior border rounded with almost no indentation, anal groove extends more towards the apical disc, the posterior ambulacra are more divergent and the test is more tumid with the summit eccentric to the front.

E. texanus Clark¹⁶ from the Austin Chalk (Upper Cretaceous) of Texas is posteriorly more rounded, its summit and apical disc are not central, the superior face slopes unequally to the front and behind.

E. angustatus Clark¹⁷ from the Commanche series of Texas is easily distinguished from the present species by its higher test, apical disc anteriorly eccentric and summit behind the middle.

E. ammonis Fournau¹⁸ from the Turonian of Egypt resembles this species to a certain extent, but the former has its test ambitally less tumid, and centrally more raised; the anal groove is relatively shorter, the poriferous zones are narrower and the apical disc a little more anterior.

Hemiasster (Mecaster) chirakhanensis sp. nov.

Plate XXV, Fig. 4

1937 *Hemiasster cf. thomasi* Peron and Gauthier, *Proc. Ind. Acad. Sci.*, Ser. B, Vol. VI, No. 1, p. 65, pl. VI, fig. 2.

Description—The test is sub-trigonal with the greatest width at $\frac{1}{4}$ length from the front and rapidly narrowing behind. The superior face rises gradually from the deeply indented front attaining the maximum height between the $\frac{2}{5}$ and $\frac{1}{3}$ length from behind. The posterior face is rather steeply truncated. The periproct is situated towards the top of a very feebly concave posterior face. The inferior face is very weakly inflated.

Pores of the odd ambulacre are oval, placed in chevrons with a small granular swelling between the pair. Scrobiculed primary tubercles rise in an internal row, up to about half the length of the poriferous zones, gradually dying out towards the apex. The ambulacral plates carry two horizontal rows of minute granules along their sutures. The paired petals are placed in deep grooves. The pore-pairs are oblique at the proximal ends, straight in the middle region passing into chevrons towards the distal ends. A horizontal line of minute granules passes dorsally to the pore-pairs. The

¹⁵ *Pal. Fr.*, p. 405, pl. 958, figs. 1-4; *Ech. Sarthe*, p. 189, pl. 32, figs. 10-13; *Synopsis*, p. 259.

¹⁶ *John Hop. Uni. Circ.*, 10, p. 76; *ibid.*, 12, p. 52; *Bull. U.S.G. Surv.*, 97, p. 62, pl. 26, fig. 2; *Memo. U.S.G. Surv.*, 54, p. 70, pl. 28, fig. 2.

¹⁷ *Ibid.*, p. 89, pl. 27, fig. 2.

¹⁸ *Cat. Invert. Egypt*, No. 2, p. 49, pl. 4, figs. 8-9.

posterior petals are three-fourths of the length of the anterior ones. Interporiferous zones are narrower than the poriferous ones. The largest of the specimens has 36 pore-pairs in the posterior petals and 42 in the anterior ones.

The peristome is situated at about one-fourths of the length from the front. The peripetalous fasciole crosses the unpaired ambulacre just above the ambitus and runs smoothly across the lateral interambulacres.

The apical disc is a little behind the middle, rather broad with four perforated genital plates. The madreporite is on the right anterior genital plate extending posteriorly so as to separate the posterior genital plates. The ocular plate III penetrates between the genital plates 2 and 3.

Tubercles are scrobiculed, dispersed without any order and become wider spaced on the inferior face.

Comparison—From the associated *H. heberti* (Coquand),¹⁹ the present species can be distinguished by its posterior face narrower, its test more trigonal, broadest more anteriorly and less tumid in the front, the posterior petals relatively shorter and less divergent, and the apical disc more sub-central.

As compared to *H. semi-cavatus* Peron and Gauthier²⁰ from the Turonian of North Africa, the present species has its test more constricted behind, the superior face is less elevated, the anterior groove is more developed, the paired petals are more unequal and the distal ends of the anterior pair are less broad.

H. thomasi Peron and Gauthier²¹ from the Senonian of Algeria is a much bigger race, its inferior face is more inflated and the superior face is more elevated and highest at the apical disc, the test is relatively broader and the posterior petals are a little longer.

Hemiasiter (Mecaster) heberti (Coquand)

1878. *Hemiasiter heberti* (Coquand), *Ech. foss. Alg.*, fasc. 4, p. 129, pl. VII, figs. 1-3.

1898. *H. heberti* (Coquand), *Mem. Inst. Egypt*, tome III, fasc. VII, p. 629.

¹⁹ *Ech. foss. Alg.*, fasc. 4, p. 129, pl. 7, figs. 1-3; *Bull. Inst. Egypt*, (4), 2, p. 51, pl. 2, figs. 14-15; *Mem. Inst. Egypt*, 3, fasc. 7, p. 629; *ibid.*, 6, fasc. 3, p. 117, pl. 6, figs. 24-25; *Cat. Invert. Egypt.*, No. 2, p. 75, pl. 7, figs. 1-2 and 6; *Mém. Soc. G. Fr.*, 16 (n.s. 7), pt. 4, p. 12.

²⁰ *Ech. foss. Alg.*, fasc. 6, p. 71, pl. 4, figs. 1-3; *Mém. Soc. G. Fr.*, 16 (n.s. 7), pt. 4, p. 126.

²¹ *Ibid.*, p. 137; *Ech. foss. Alg.*, fasc. 7, p. 72, pl. 4, fig. 1; pl. 3, fig. 8.

- 1901 *H. artini* Gauthier, *Bull Inst Egypt*, ser 4, vol II, p. 51, pl. II, figs 14-15.
- 1909 *H. artini* Gauthier, *Mem Inst Egypt*, vol VI, fasc II, p 117, pl VI, figs 24-25
- 1914 *H. heberti* (Coquand), *Cat Invert foss Egypt*, p 75, pl VII, figs 1-2 and 6
- 1932 *H. heberti* (Coquand), *Mem Soc Geol Fr*, tome 16 (n s 7), pt. 4.

The specimens from the Bagh Beds agree with the Algerian type, except that they are relatively a little less in width and in height

Hemiaster (Mecaster) saadensis Peron and Gauthier

- 1878 *Hemiaster saadensis* Peron and Gauthier, *Ech foss Alg*, fasc IV, p 125, pl VI, figs 1-4
- 1932 *H. saadensis* Peron and Gauthier, *Mem Soc Geol Fr*, Mem 16, n s 7, fasc 4, p 138

As compared to the type from Algeria, the Indian specimens have a very feeble carination on the odd inter-ambulacre which is not observed in the type

Discussion and Conclusions

The accompanying table shows, with the new additions and corrections, the vertical distribution and the affinity relations of the echinoid species from the Bagh Beds with those from other countries. The species reported upon in the present paper are marked with an asterisk

Among the new species dealt with in this paper, *Hemiaster heberti* (Coquand) and *H. saadensis* Peron and Gauthier are known from the Cenomanian of North Africa, while *H. chirakhanensis* sp. nov. is related to *H. thomasi* Peron and Gauthier from the Senonian of Algeria. All these species belong to the group of *Mecaster* Pomel,²² which appears first in the Cenomanian. Of the echinobrissi, *Echinobrissus malwænsis* sp. nov. is related to *E. nicoleti* d'Orb. from the Urgonian of France, and appears, as already remarked, to be more advanced than the French species. *E. chirakhanensis* sp. nov. has its nearest ally *Nucleolites similis* Desor from the Cenomanian of Sarthe, while *E. rajnathi* sp. nov. is related to *E. kœchlini* d'Orb. from the Albian of France.

²² *Essais. Rais.*, p. 501.

TABLE I

Table showing the Vertical Distribution and Affinity Relations of the Echinoids from the Bagh Beds

No.	Species from the Bagh Beds	Related species with stratigraphical position	Nodular Limestone	Lower Coralline Limestone	Deola Chirakhan Marl	Upper Coralline Limestone
1	<i>Dorocidaris namadica</i> Duncan	<i>Doro julieni</i> Gauthier, Aptian of Algeria			×	×
2	<i>Salenia keatingsi</i> Fourtau	<i>S. mamillata</i> Cotteau, Aptian of France			×	
3	<i>S. mathuri</i> Chiplonker	<i>S. cylindrica</i> Arnaud, Aptian Albian of France			×	
4	<i>Cyphosoma namadicum</i> Fourtau	<i>C. peroni</i> Cotteau, Barremian Aptian of France and Switzerland		×	×	×
5	<i>Orthopora indica</i> Duncan	<i>O. repellens</i> Desor, Barremian Aptian of France, Portugal and Switzerland			×	
6	<i>Diplopodia (Tetragramma) aff. micropyga</i> Fourtau	<i>D. micropyga</i> Fourtau, lower Cenomanian of Egypt			×	
7	<i>Echinobriacus haydeni</i> Fourtau	<i>E. eddiacensis</i> Gauthier, Aptian Albian of Algeria and Tunis	×	×	×	×
* 8	<i>E. malwanensis</i> sp. nov.	<i>E. nicolleti</i> d'Orb., Urgonian of France			×	
* 9	<i>E. chirakhanensis</i> sp. nov.	<i>Nucleolites similis</i> Desor, Cenomanian of Sarthe		?	×	×
* 10	<i>E. rajnathii</i> sp. nov.	<i>E. kachims</i> d'Orb., Albian of France	?		×	•
11	<i>Hemaster fourtaui</i> Chiplonker	<i>H. luynei</i> Cotteau, Cenomanian of Palestine	×	×	×	×
12	<i>H. (Proraster) sub similis</i> (Fourtau)	<i>H. morganii</i> Cotteau and Gauthier, Senonian of Persia	×	×	×	×
13	<i>H. holo-ambitatus</i> Chiplonker	<i>H. asterius</i> Forbes, Albian of Yonne and Folkstone, <i>H. rufus</i> Lambert, Senonian of Algeria	×	×	×	×
14	<i>H. (Mecaster) maslei</i> Peron and Gauthier	<i>H. maslei</i> Peron and Gauthier, Cenomanian of Algeria, Tunis and Egypt		×	×	×
* 15	<i>H. (Mecaster) chirakhanensis</i> sp. nov.	<i>H. thomasi</i> Peron and Gauthier, Senonian of Algeria		×	×	×

TABLE I—(Contd)

No	Species from the Bagh Beds	Related species with stratigraphical position	Nodular Limestone	Lower Coralline Limestone	Deola-Chirakhan Marl	Upper Coralline Limestone
*16	<i>H. (Mecaster) heberti</i> (Coquand)	<i>H. heberti</i> (Coquand). Cenomanian of Algeria and Egypt	.		×	
*17	<i>H. (Mecaster) saadensis</i> Peron and Gauthier	<i>H. saadensis</i> Peron and Gauthier, Cenomanian of Algeria			×	×

To discuss fully the evidence now available after a detailed study of all the echinoids thus far known to occur in the Bagh Beds, it will be seen that out of the seventeen species, *Hemaster heberti* (Coquand), *H. saadensis* Peron and Gauthier and *H. meslei* Peron and Gauthier²³ mark the Cenomanian horizon in North Africa. *Diplopodia aff. microphyga* Fourtau,²⁴ *Hemaster fourtau* Chiplonker²⁵ and *Echinobrassus chirakhanensis* sp. nov. have distinct affinities with Cenomanian species from Egypt, Palestine and France respectively. *H. holo-ambitalis* Chiplonker²⁶ is related to the species which are known to occur in the Albian and in the Senonian of the Anglo-Belgian basin, while *H. sub-similis* (Fourtau)²⁷ and *H. chirakhanensis* sp. nov. have their relations in the Senonian of Persia and Algeria respectively. The rest of the eight species as remarked before, are related to eo-cretaceous forms and appear to be ancestral to meso-cretaceous species.²⁸ With these relations, in conjunction with the Lower Chalk (Cenomanian) as the upper age limit for the genus *Diplopodia* McCoy,²⁹ and Cenomanian³⁰ as the lower limit for the groups of *Proraster*, *Mecaster* and *Integraster* to which six, out of seven species of *Hemaster*, belong, the echinoids from the Bagh Beds must be regarded to mark the Cenomanian, and very probably the lower Cenomanian age.

As regards the correlation of the different constituents of the Bagh Beds with those of the Cretaceous series of South India, the vertical distribution of

²³ *Ech. foss. Alg.*, fasc. 4, p. 102; *Ech. Tunisie*, p. 12; *Cat. Invert. Egypt*, No. 2, p. 81.

²⁴ *Ibid.*, No. 5, p. 26; *Proc. Ind. Acad. Sci.*, (B), 6, p. 62.

²⁵ *Ibid.*, p. 64.

²⁶ *Ibid.*, p. 64.

²⁷ *Rec. G.S.I.*, 49, p. 50.

²⁸ *Ibid.*, p. 53; *Proc. Ind. Acad. Sci.*, (B), 6, p. 66.

²⁹ *Essais. Rasse.*, pp. 185-87.

³⁰ *Ibid.*, pp. 501, 504, 506.

the echinoid species dealt with in the present paper, as can be seen clearly from the accompanying table, only goes to support the conclusion already arrived at,⁸¹ namely the different constituents of the Bagh Beds must be assigned to a single geological age, the observed differences being due to only lithological facies rather than to geological time

Acknowledgments

In conclusion, I express my great indebtedness to the late Prof K K Mathur for kindly putting at my disposal his collection of fossils from the Bagh Beds, which forms a part of the material for the present work. Very sincere thanks are due to Dr Raj Nath for kindly going through the manuscript of this paper and for his valuable criticism. My grateful thanks are due to the Director, The Geological Survey of India, for the kind permission to work in the Survey Museum and Library. I take this opportunity of thanking the Dhar and Indore Durbars for the kind permission to collect fossils from their territories and for the facilities given during the field work.

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⁸¹ *Proc Ind. Acad Sci*, (B), 6, p 67, *Mém G.S.I*, 21, pt 1, pp 39, 41, 44, 48, *Curr. Sci*, 4, p. 322.

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EXPLANATION OF THE PLATE

N B.—All figures are natural size.

- FIG 1 —*Echinobrius malicensis* sp. nov., (a) superior view; (b) lateral view (B.H.U No E/14)
- FIG. 2.—*Echinobrius rajnathi* sp. nov., (a) superior view; (b) lateral view (B.H.U, No. E/7).
- FIG. 3 —*Echinobrius chirakhanensis* sp. nov., (a) superior view; (b) lateral view (B.H.U. No. E/8).
- FIG. 4.—*Hemaster chirakhanensis* sp. nov.; (a) superior view; (b) lateral view (B.H.U. No. E/12).



3a



2a



1a



3b



2b



1b



4a



4b

ROOT DEVELOPMENT OF CERTAIN OILSEED CROPS OF THE PUNJAB

BY ALI MOHAMMAD, M SC (AGRI), L AG

Botanist for Oilseeds, Agricultural Research Institute, Lyallpur

AND

ABDUR RASHID KHAN, B SC (AGRI)

Agricultural Assistant, Oilseed Section, Lyallpur

Received December 9, 1938

(Communicated by Mr B N Sastri, M A (), A I C (), A I T I C ())

I Introduction

Of the oilseed crops grown in the Punjab, *toria* (*Brassica napus* L. var *dichotoma* Prain) and *sarson* (*Brassica campestris* L. var *sarson* Prain) are by far the most important. *Toria* is grown chiefly in the canal colonies and occupied an area of 418,256 acres in the year 1936-37, out of which 379,080 acres were irrigated. *Sarson* is sown almost all over the Province. The area under this crop is not separately recorded in the official records but is given with *raya* or *rai* (*Brassica juncea*) and *taramira* (*Eruca sativa*) which is generally regarded as a *Brassica* substitute. The total area under the last-mentioned three crops during the year 1936-37 was 563,647 acres, out of which only 80,770 acres were irrigated. The plants of the above four species differ materially above ground and are usually grown under markedly different conditions in the Province. *Toria* is the most early ripening of these and seldom succeeds without irrigation. *Sarson* and *raya* or *rai* are medium in maturity and are usually sown on unirrigated lands. *Taramira* is generally grown in extremely dry tracts of the Province and is one of the most drought resistant of the cultivated crops known at present.

The results of some of the breeding investigations carried out on these crops in the Punjab have already been published from time to time and are referred to in the last publication by Ali Mohammad and Sikka (1937). A number of improved types and selections of the four species referred to above have been evolved. The present studies relate to the work done, in connection with the root development of typical varieties of the species concerned. It may be mentioned that a thorough knowledge of the root habit is of very great value in the improvement of crops by selection. The work carried out by various investigators in recent years shows that although

general form of root is very responsive to environmental factors, yet, it is also governed by heredity. The Howards and Abdur Rahman Khan (1915, 1917 and 1924) have published an interesting account of the root system of several crops such as gram, wheats, linseed grown at Pusa. Their investigations indicate that in the unit species of a crop growing under uniform conditions quite distinct kinds of root-systems develop in different unit species or types. In other species definite differences in root system have been shown by Ali Mohammad and Deshpande (1929) with chillies, by Venkatraman and Vittal (1928) with sugarcane seedlings and by Jean (1928) with peas. The present investigations into the root-habits of the oilseed crops referred to above are intended to add further to our knowledge of this subject.

II Material and Method

The following typical varieties of the above-mentioned four species were under study —

- (i) *Tona* selection A
- (ii) *Sarson*—
 - (a) Brown selection A
 - (b) Yellow type L-1
 - (c) Yellow type L-5
- (iii) *Raya*—
 - (a) Type O B 1 (L-18), a comparatively early ripening and tall growing type
 - (b) Type L-13, a very late ripening and very tall growing type
- (iv) *Taiamira* selection A

These types were grown in the year 1937-38 at the Oilseed Breeding Station, Lyallpur, under the same environmental conditions with a view to obtain comparative root differences between them which may be attributed to heritable behaviour. The surface soil of the plot in which the plants were grown was medium loam. A fairly compact layer of *kankar* (small particles of calcium carbonate) of about one foot thickness was observed in the subsoil at a depth of about four feet from the soil surface. Another similar layer was found at nine feet depth. Before sowing, the land was thoroughly well prepared by giving three ploughings. All the types were sown on the 1st of October in rows 5 ft apart and there were 30 plants set out in each row for the purpose of their root studies. The plot received two irrigations during the season. Three representative plants of each of these types or even four in some cases when considered necessary were examined for the purpose of these studies at two stages during the growing season, *viz*,

(a) Sixty days after sowing and

(b) At maturity

The usual trench method of excavation was employed and trenches about 4 ft wide and sufficiently deep to enable trenching of deepest located roots were dug about 3 ft away from the plants. The root systems of the plants were then exposed by gently spraying away the soil to uncover the root-systems by means of a Knapsac Sprayer. Data regarding the position, extent, degree of branching were recorded and pencil drawings of root-system of each type were made (Figs 1 and 2). A brief description of the root-system of each type is given with such details as are considered necessary to bring out clearly the outstanding differences among the

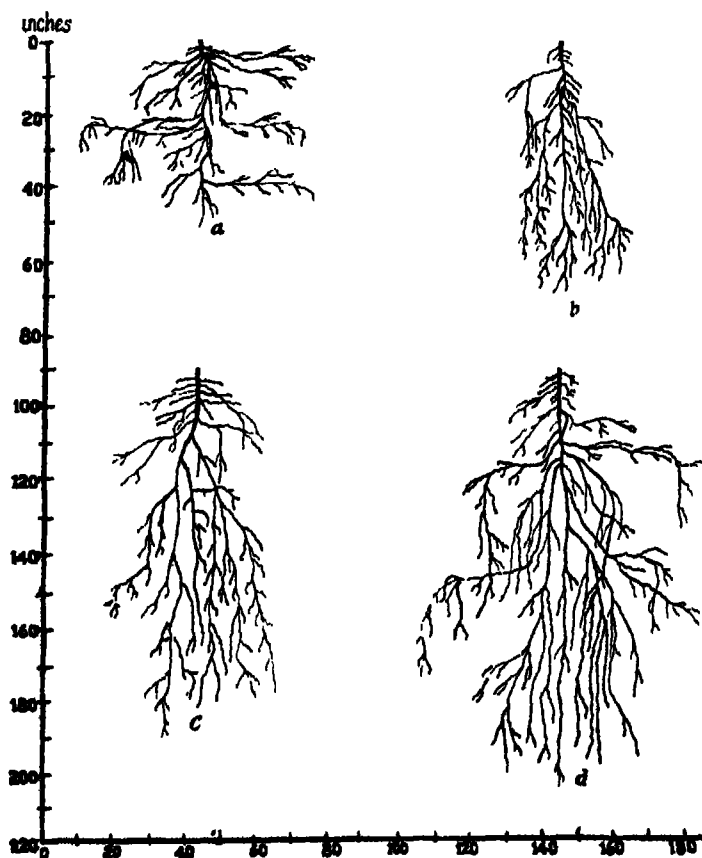


FIG 1

Root Systems at Maturity

a—*Toria A*

b—*Sarson brown A*

c—*Sarson yellow L-1*

d—*Sarson yellow L-5*

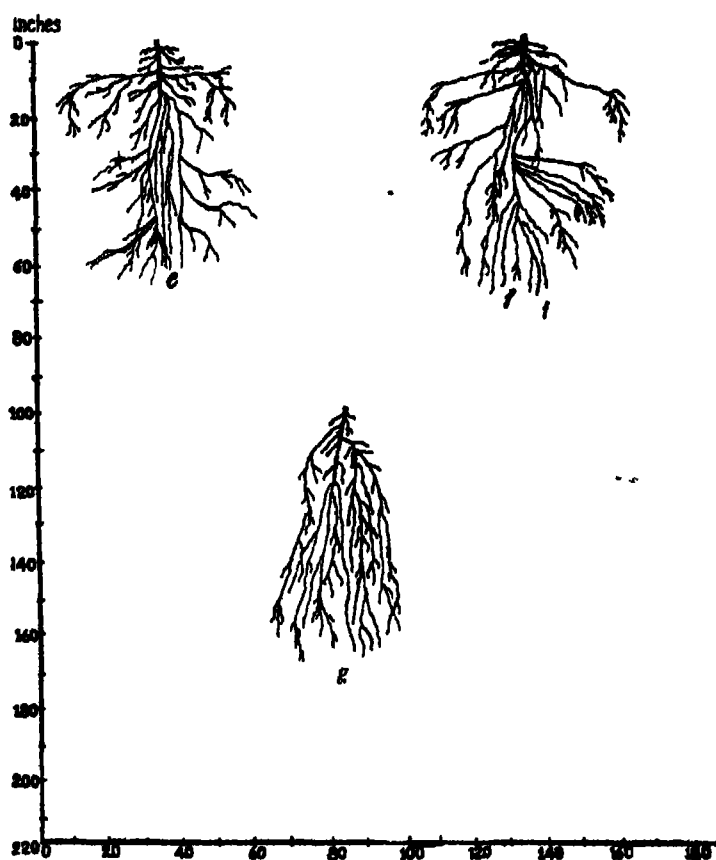


FIG. 2

Root Systems at Maturity

e—(Raya L-18)

f—(Raya L-18)

g—(Taramura A)

various types. The important above ground characters of the plants examined are also given in order to make a comparison between root and shoot growth as far as possible.

III Description of Root-Systems

(i) Toria selection A—

The plants excavated 60 days after sowing had 4 to 6 branches each and had an average height of 45". The tap root was 1 2" thick near the soil surface and went down to a depth of 38" to 43". There were a large number of prominent secondary roots which were uniformly distributed laterally but they did not go deep into the subsoil.

At the time of second digging, *i.e.*, at maturity, the plants were 45" to 50" high and possessed 7 to 9 branches. The tap root was 1.5" thick and penetrated into the soil to a depth of 48" to 56". The working depth of the root-system, *i.e.*, the depth to which majority of roots penetrated was about 38" with a lateral spread of about 60". The root-system, on the whole, was comparatively shallow but had a large number of well-developed secondary roots having an extensive lateral spread (Fig 1-a)

(ii) *Sarson*—

(a) *Brown selection A*—At the time of first digging the plants were 35" to 40" high and possessed 4 to 6 branches each. The tap root was about 1.5" thick near the soil surface and went much deeper than that of *toria*. There were comparatively fewer well-developed secondary roots but they were found to grow much deeper into the ground.

At the time of maturity, the plants had reached an average height of 53" and possessed a fairly large number of branches. The tap root was nearly as thick as that of *toria* but went very deep into the subsoil. There were a few prominent secondary roots but a large number of thin secondary and tertiary roots. The root-system, on the whole, was comparatively deep and vigorous having rather limited lateral spread (Fig 1-b)

(b) *Yellow type L-1*—At the time of first excavation of roots the plants of this variety were 45" to 50" in height and possessed 5 to 6 branches each. The tap root was almost as thick as that of brown *sarson* and went to a depth of 65" to 74" into the subsoil. There were a fairly large number of thick and thin secondary roots which were well distributed throughout the feeding zone of the root-system.

At the time of maturity, the plants grew 55" to 60" high and had 8 to 10 branches each. The tap root did not grow much thicker but went much deeper into the subsoil. The secondary and tertiary roots were large in number and also grew deep into the subsoil with a working depth of about 80". The root-system, on the whole, was vigorous with extensive working depth as well as extensive lateral spread (Fig 1-c)

(c) *Yellow type L-5*—The plants of this variety were 50" to 55" high and had 5 to 6 branches each at the time of their first examination. The tap root was medium in thickness and went deep into the subsoil. The secondary and tertiary roots were large in number and most of them went deep into the subsoil.

At the time of final digging, the plants had attained a height of 60" to 65" and possessed about 10 branches each. The tap root was medium in thickness but very great in length. The number of secondary thick and

thin roots was extremely large and they had a working depth of about 100" and a lateral spread of about 76" The root-system, on the whole, may be described as extremely vigorous and deep (Fig 1-d)

(iii) *Raya*—

(a) *Type O B 1 (L-18)*—The plants of this variety at the time of first digging were 48" to 54" high and possessed 7 to 8 branches each The tap root was about 1 5" thick and went down to a depth of about 50". There were a few prominent secondary roots but a large number of thin secondary and tertiary roots.

At maturity, the plants were found to grow about 100" tall with a large number of shoots The tap root was found to have grown very thick and penetrated fairly deep into the subsoil The number of secondary roots was fairly large and most of them went fairly deep into the subsoil The roots also had an extensive lateral spread The root-system, on the whole, was very vigorous (Fig 2-e)

(b) *Type L-13*—At the time of first digging the plants had just started their growth and were only 20" to 23" high The tap root was very thin and 20" to 25" in length There were only a few short secondary roots

At the time of second digging, i.e., at maturity, the plants were found to have grown extremely tall and bore a large number of prominent secondary roots going deep into subsoil and giving off branches mainly at the lower depths The lateral spread of the roots was 40" to 50" The root-system, on the whole, was deep and vigorous having extensive lateral spread (Fig 2-f)

(iv) *Taramira selection A*—

At the time of first examination the plants were 28" to 33" high and possessed about 16 branches each The tap root was thin and went fairly deep into the subsoil There were a few prominent secondary roots but comparatively large number of secondary and tertiary roots

At the time of maturity, the plants were 46" to 52" high and possessed a large number of branches The tap root was comparatively thin but went very deep into the subsoil There were only a few prominent secondary roots but a very large number of thin roots Very few roots were, however, produced near the soil surface The root-system, on the whole, was deep and vigorous having limited lateral spread (Fig 2-g)

IV Conclusions

The data obtained from the results of investigations quoted above are summarised (Table I) showing the comparative differences in the root-system of the types studied as compared to their top growth. Judging

TABLE I
Data regarding the comparative root and shoot development of different
Oleiferous Brassicæ and Taramira

Name of crop	No of type or selection	First excavation (60 days after sowing)										Second excavation (at maturity)									
		Root						Shoot				Root						Shoot			
		Thickness of tap root in inches	Maximum in inches	Depth of root system in inches	Lateral spread in inches	Average No of roots per plant		Depth at which well developed secondary roots appear (inches)	Height in inches	No of branches	Thickness of tap root in inches	Maximum in inches	Depth of root system in inches	Lateral spread in inches	Average No of roots per plant		Depth at which well developed secondary roots appear (inches)	Height in inches	No of branches		
						Thick	Thin								Thick	Thin					
Toris	A	1 2	42	40	19	2	14	3	45	4	1 5	48	38	60	8	5	2	48	9		
Brown sarson	A	1 5	60	50	16	3	7	7	39	5	1 6	68	55	35	4	16	6	53	8		
Yellow sarson	L-1	1 5	70	60	32	7	20	5	47	6	1 5	100	82	43	12	30	7	57	9		
Do	L-5	1 2	60	56	24	7	18	8	51	5	1 5	114	100	76	13	36	12	62	10		
Rays	L-18	1 5	51	43	28	3	14	12	50	7	2 3	60	50	46	8	25	6	99	10		
Do	O B 1 L-13	0 6	22	17	8	3	12	2	21		2 5	69	67	56	10	35	5	102	9		
Taramira	A	0 5	54	43	16	2	7	9	30	16	0 9	61	52	32	5	24	9	48	15		

from the results obtained the four species under observation can be grouped into three distinct classes according to the development of their root-system and their adaptation to certain agricultural conditions. These three classes are specified below —

Class I —Root-system having limited working depth and extensive lateral spread

Of the above seven types of plants, *toria* represents this class. This type of root-system is more or less a surface feeder and is evidently suited for irrigated conditions. This also explains why the cultivation of this crop is restricted to irrigated tracts.

Class II —Root-system having extensive working depth and limited lateral spread

Brown *sarson* and *taramira* apparently belong to this group and they are evidently better suited to conditions where there are no means of irrigation available and the water has to be drawn by plants from the subsoil. Ordinarily, such crops are not likely to be benefited much by surface irrigation. This appears to be the main reason for their cultivation having been restricted to *barani* (rain-fed) areas in the Province.

Class III —Root-system having extensive working depth and extensive lateral spread.

Both the types of yellow *sarson* and both the types of *raya* included in the studies fall under this group. All these types seem to be admirably well adapted to both irrigated and unirrigated conditions so far as their root growth is concerned and it follows that although such types may be expected to do well under *barani* conditions, yet, they seem to be capable of flourishing better under artificial irrigation.

In conclusion, it may be mentioned that the grouping of various types of plants in accordance with their root growth on the above lines is considered to be a matter of great practical importance in so far as the successful cultivation of these crops under different conditions in the Province is concerned. Besides the above types, a large number of other types of these crops have been evolved in recent years. Possibly, a study of their root systems may lead to the discovery of certain other slightly different types of root systems as distinct from those described above.

All the same, it seems necessary that the various available types should be examined and arranged in suitable groups with regard to their habit of root growth. The investigations will be extended further as far as possible with a view to selecting types of these crops suitable for different localities.

V Summary

1 Root development of representative types of *toria*, *sarson*, *raya* or *rai* and *laramira* have been studied at two stages of their growth

2 The various types under study have been placed in the following classes as regards their habit of root growth —

- (a) Root-system having limited depth but extensive lateral spread.
- (b) Root-system having extensive depth but limited lateral spread
- (c) Root-system having extensive depth and extensive lateral spread.

3 Suitability of different types of root-system to different conditions has been discussed and it has been shown that —

- (a) *Toria* is essentially suited for irrigated tracts
- (b) Brown *sarson* and *laramira* are best suited for *barani* (rain-fed) areas
- (c) Types of yellow *sarson* nad *raya* under study are considered likely to succeed under both irrigated and unirrigated conditions

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ON THE EXTERNAL GILLS OF *ACENTROGOBIUS VIRIDIPUNCTATUS* (DAY)

BY S JONES

(From the University Zoological Research Laboratory, Madras)

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Introduction

THE presence of true external gills in *Acentrogobius viridipunctatus* has been noticed while working out its development and this is the first instance of their known occurrence in Teleosts (Jones, 1937 a) *A. viridipunctatus* is found in fair abundance in all the brackish-water areas in Madras. At Adyar they are found inside oyster shells and crevices between stones, where their eggs are also found with the male guarding and aerating them. The breeding habits and development of this form are described elsewhere (Jones, 1937 b).

Among the vertebrates external gills are known to be present only in Fishes and Amphibians and they are all mostly embryonic and larval respiratory organs except in some of the latter where they persist and function in the adult also. Goodrich (1930) distinguishes two kinds of external gills, viz., external gill filaments and true external gills. Of these the former are mere filamentous elongations of the gill lamellæ and are known to be present only among the embryos of Elasmobranchs, and the larvæ of a few Teleostomes. Though common in Elasmobranchs they are very rarely found among Teleostomes, the best known examples among them being the two African fishes *Gymnarchus* and *Heterotis* described by Budgett (1901).

A true external gill is described by Kerr (1919) as follows " The typical external gill is a projection from the surface of the body on the outer side of a visceral arch. It consists of a core of mesenchyme with a covering of ectoderm, it is traversed by a vascular loop consisting of the main aortic arch which passes out to its tip and then doubles back, and it commonly has a pinnate form, paired projections growing out so as to increase its respiratory surface. It is provided with muscles by which the possessor is able to flick it sharply backwards so as to renew the water in contact with it " (p 154). It is in this sense that the term is used in this paper.

True external gills are, however, absent in Cyclostomes and Elasmobranchs. Among the Ganoids they are present only in the larvæ of *Polypterus* (Budgett, 1901 and Kerr, 1907) as a pair of bi-pinnate structures, one on each side of the hyoidean arch. It is supported at the base by a short cartilaginous rod, though a skeletal support of any kind is absent in any other external gills of the living forms. In Dipnoi, the external gills are present as four pairs of pinnate structures in the larvæ of *Protopterus* (Budgett, 1901) and *Lepidosiren* (Kerr, 1900) on the outer side of the four branchial arches. In *Protopterus* they are usually retained in a much reduced condition even in the adult. In *Lepidosiren* they are absent in the adult but during the late larval period the four gills on each side come close together and fuse at the base (Kerr, 1900). External gills are to my knowledge unknown in Teleostei and those in *A. viridipunctatus* described in this paper are the first of their kind.

In Amphibia they are well represented in all the groups. They are either pinnate or arborescent structures arranged outside the third, fourth and fifth visceral arches. In Apoda they are known only as embryonic respiratory organs (*Ichthyophis*—P and F Sarasin 1889 and *Hypogeophis*—Brauer, 1899). In Urodela and Anura they are larval organs except in the Perennibranchiata where they persist and function in the adult also. In those forms where there is no aquatic larval life the external gills are either absent or present only as embryonic respiratory organs.

The balancers of Urodela are considered by some (Kerr, 1919, Gadow, 1920) as modified external gills, but Goodrich (1930) is opposed to this view and has suggested reasons for considering them as homologous with the larval adhesive organs of Anura. As mentioned in my paper on the Cement organs in *Etiopplus maculatus* (Jones, 1937c) the fact that the Cement organs of larval *Acipenser* get modified into barbels, may be taken as an additional evidence in support of this view.

Development of the External Gills

Blood circulation is established in the embryo when it is about three days old. Gill slits and opercular folds are absent at this stage and the aortic arches are not provided with afferent and efferent branchial vessels, the four vessels of the branchial arches being directly connected to the dorsal aorta. In front of the first pair of branchial aortic arches the ventral aorta divides into the two large hyoidean arteries which travel along the hyoid arch and sink below the eyes after which their course is difficult to follow. In front of the hyoidean arch the ventral aorta is continued into a small vessel which divides into the two mandibular aortic arches.

When the embryo is three and a half days old a median projection is formed outside the base of the hyoid arch below the place where the ventral aorta divides into the two hyoidean arteries. This projection is a rudiment of the fused portion of the external gills. As it grows, the commencement of the aortic arch on each side gets pulled into it with the result, the hyoidean arteries are thrown into vascular loops and in a four days old embryo the median gill rudiment bifurcates. More blood is sent into these than through any other arterial arch. When the embryo is five days old the anterior extremities of the external gills reach as far as the mouth. In the six days old embryo the external gills grow long enough to bend round the snout and in some cases reach the eyes and hatching usually takes place about this time.* The animal within the egg case occasionally flicks the organ gently. Embryos not undergoing normal development have poorly developed external gills. Such seldom hatch out and even if they do so, do not survive long.

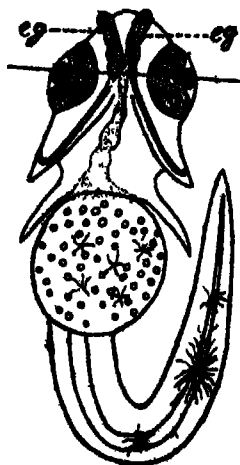


FIG. 1

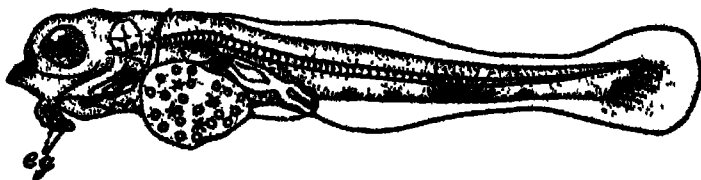


FIG. 2

* For the external features in the embryonic development, see Jones, 1937 b.

The stages in the development of the external gills are diagrammatically represented in Fig 12 A-E

Fig 1 is a ventral view of a six days old embryo showing the well-developed external gills which have contracted when coming into contact with water. On hatching the gills shrink into two small structures behind the lower jaw (Fig 2). The contraction of the blood vessels is so great that the flow of blood to them or from them is completely stopped. The organs are functional only during the embryonic period.

Structure

It has already been said that in the early embryo the external gills take their origin from the base of the hyoidean arch. The arrangement of the cartilaginous elements in the region of the external gills in the young larva could be made out from Figs 3 and 4. At the time of hatching the hypohyals, hypobranchials and the basihyal are in the stage of pro-cartilage and are not distinctly differentiated.

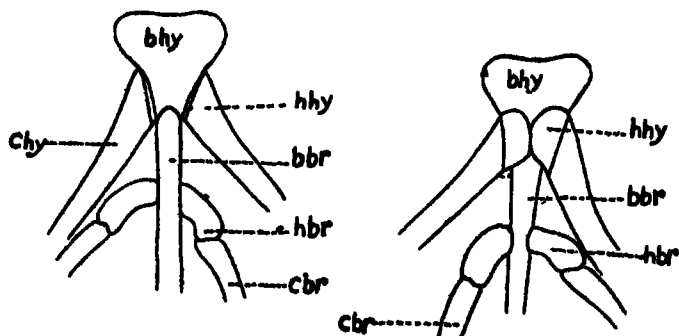


FIG 3

FIG 4

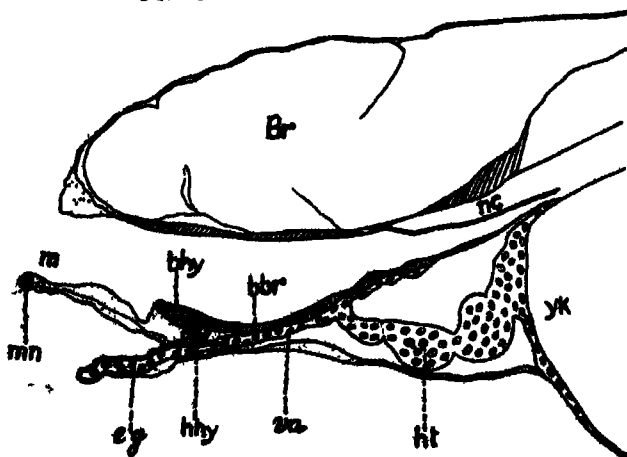


FIG. 5

In a longitudinal median section of a newly hatched larva (Fig 5) the fused portion of the external gills can be seen rising just in front of the hypohyals and ventral to the basihyal. The ventral aorta is continued anteriorly along the ventral side of the basibranchial and the hypohyals and then enters the external gills (*eg*) where it divides into the hyoidean arteries

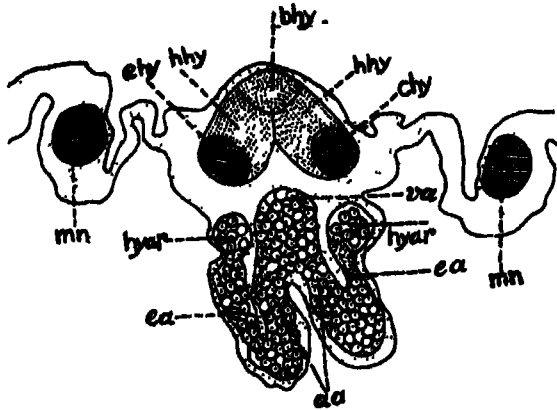


FIG 6

The hyoidean arteries returning from the gills, after the formation of the two vascular loops, enter the proximal portion of the ceratohyals along which they are continued. The cartilages in relation with the fused basal portion of the external gills are partly the two ceratohyals. Judging from their position, probably attachment to the hypohyals which are not yet fully chondrified at this stage, exists. Fig 6 is a section passing through the shrunken external gills filled with blood corpuscles. Above the external gills can be seen the two ceratohyals (*chy*) with pro-cartilaginous hypohyals (*hhy*) in between. Above them is the posterior end of the basihyal (*bhy*). In the

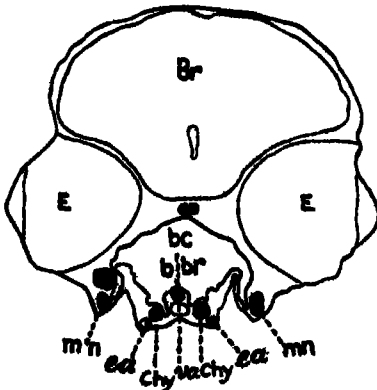


FIG. 7

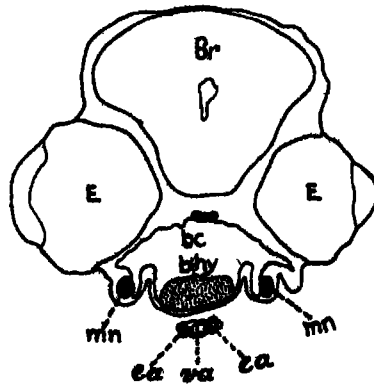


FIG. 9

middle, below the hypohyals is the anterior continuation of the ventral aorta (*va*) which after a short distance divides into the two hyoidean arteries which give rise to the two vascular loops of the external gills. The inner vessels constitute the afferent arteries (*aa*) and the outer, the efferent arteries (*ea*) of the external gills. The latter enter the hyoid arch again at the basal end of the ceratohyals (*chy*)

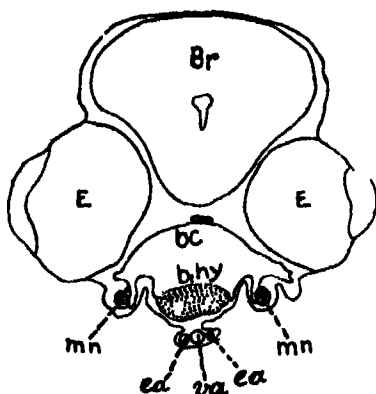


FIG 8

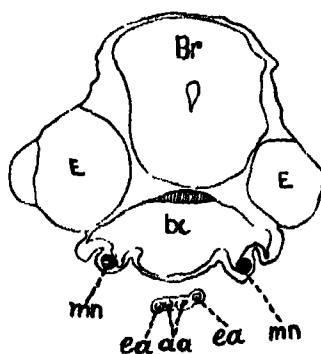


FIG 10

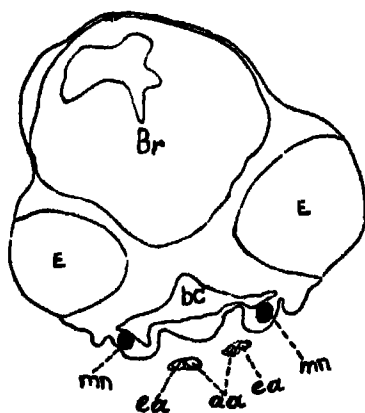


FIG 11

Fig 7 shows a transverse section through the head just behind the external gills. Below the basibranchial (*bb*) is the anterior continuation of the ventral aorta (*va*) before it enters the external gills. Below the ceratohyals (*chy*) are the hyoidean arteries (*ea*) which double back from the external gills after the formation of the loops. In Fig 8 the section passes a little more to the front and shows the median ventral aorta (*va*) and the efferent vessels of the gills. Anterior to this the fused portion of the gills

become free from the body (Fig 9). In Fig 10 the ventral aorta has just divided to give rise to the afferent arteries (*aa*), covered by an extremely thin layer of mesenchyme and ectoderm. Anterior to this (Fig. 11) the gills become separate

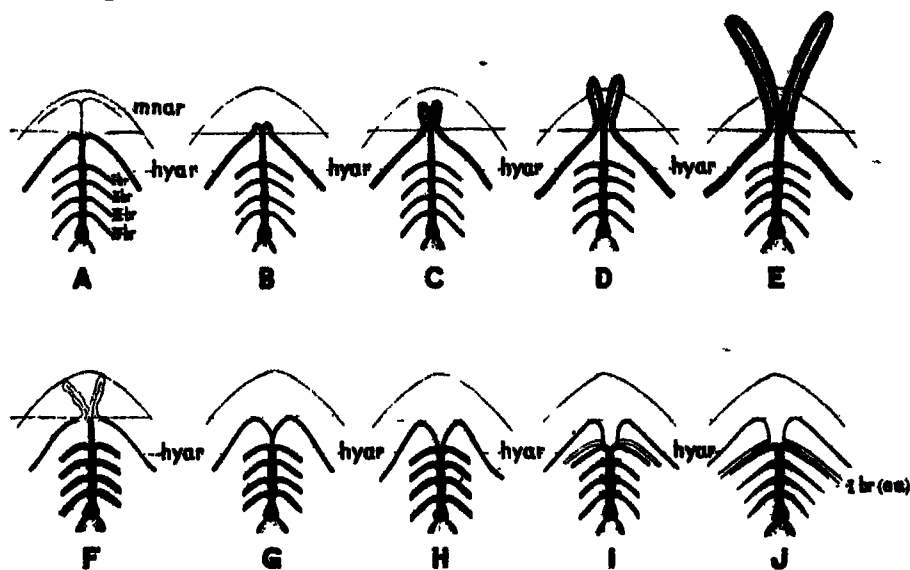


FIG. 12

Degeneration

Shortly after hatching, a little distance in front of the first branchials, a cross connection is established between the ventral aorta and the efferent vessels of the external gills, forming a short circuit, as a result of which blood supply is cut off leading rapidly to their atrophy. The cross connections are formed from pre-existing minute vessels as in *Lepidosiren* (Robertson, 1913). The quantity of blood sent through the hyoidean artery is considerably reduced. A shortening of the ventral aorta in front of the first branchials takes place with the result that the bases of the hyoidean and those of the first branchials are approximated and later fusion with one another occurs. Gradually as efferent branchials are established, the hyoidean arteries get connected to them. The changes that take place in the hyoidean arteries are diagrammatically represented in Fig 12, F-J. The external gills completely disappear by the second or the third day after hatching.

Discussion

The external gills of *A. viridipunctatus* differ from the typical ones in their simple (non-pinnate) nature and median partially united condition. Pinnate and arborescent growths are developed in the external gills for

increasing their respiratory surface. We get them in the most highly developed condition in the Perennibranchiates where they are the main respiratory organs in the adult. In Crossopterygii and Dipnoi where external gills are all larval organs, they are pinnate or bi-pinnate structures and they function for a fairly long period. In *A. viridipunctatus* the gills continue to be simple, probably a primitive feature.

The partially fused condition of the gills here has to be regarded as a secondary feature, the paired condition being the more primitive. In this connection it is interesting to note that the two vascular loops of the gills remain separate. In *Lepidosiren*, during the late larval period the four external gills of each side come closer and fuse at the base giving the appearance of a single organ with four branches. Here an approximation of the two gills of opposite sides has taken place producing a median fusion.

The view that the external gills are very ancient organs in vertebrates has been advanced by Kerr (1919), though their absence in Cyclostomes and Elasmobranchs as pointed out by Goodrich (1930) is evidence against this view. If the latter opinion is to be accepted the alternative that the gills have evolved independently in the various groups of vertebrates has to be considered seriously. Against this it has been said that the organs in question are so similar both in regard to structure and development that it is hard to believe that they can be without any phylogenetic significance. However, our present knowledge of the external gills is not sufficient to justify in arriving at any definite conclusion on this question and it is worth while looking for these organs in other Teleostean fishes.

Summary

1. The origin, structure and degeneration of the external gills of *A. viridipunctatus* have been studied.

2. They are two simple partially united structures having a median position at the base of the hyoidean arch.

3. They develop during the late embryonic period and remain functional till the time of hatching.

4. The ventral aorta is continued into the fused basal portion and the free portions are formed by the two vascular loops of the hyoidean arteries.

5. Attachment of the fused basal portion of the gills extends from the proximal portion of the ceratohyal on one side to that on the opposite side.

6. Between the blood-vessels and the outer ectoderm is a thin layer of mesenchyme.

7. The animal is able to flick gently the organs when they are well developed.

8 When the larva hatches out the gills shrink in size due to cessation of blood supply and their atrophy is completed by the third day.

Acknowledgment

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EXPLANATION OF FIGURES

LIST OF ABBREVIATIONS

aa, Afferent arteries; *bbr*, Basibranchial, *bc*, Buccal Cavity; *bhy*, Basihyal; *Br.*, Brain, *I br*, *II br*, *III. br*, *IV br*, The Branchial arches; *cbr.*, Ceratobranchial; *chy*, Ceratohyal; *E*, Eye; *ea*, Efferent Arteries; *eg*, External Gill, *hbr*, Hypobranchial, *hh*, Hypohyal, *ht*, Heart, *hyar*, Hyoidean artery; *m*, Mouth; *mn.*, Mandibular, *mar.*, mandibular artery, *nc*, Notochord; *va.*, Ventral Aorta; *yk*, Yolk

- FIG 1. Ventral view of an extracted embryo showing the external gills. The organs have shrunk into small structures. The region anterior to the line is the portion free from the body. x 28.
- FIG 2. Side view of a newly hatched larva showing the functionless external gills behind the lower jaw. x 28
- FIG. 3. The cartilaginous elements in the region of the external gills. Dorsal view. x 152.
- FIG 4. Ventral view of the above x 152.
- FIG 5. A longitudinal median section of the head of a newly hatched larva passing through one of the external gills. x 120.
- FIG 6. An oblique section passing through the external gills filled with blood corpuscles x 460.
- FIG. 7. Transverse section of the head just behind the external gills. x 115.
- FIG 8. Transverse section of the head at the base of the fused portion of the external gills x 115.
- FIG. 9. A section anterior to the one in Fig. 9. The fused portion has become free from the body. x 115.
- FIG 10. A section immediately after the division of the ventral aorta into the two hyoidean arteries x 115.
- FIG 11. A section anterior to the one in Fig 11 showing the two gills separate with the inner afferent and the outer efferent blood vessels. x 150.
- FIG. 12. A-E. Diagrammatic representation of the formation of the vascular loops of the external gills F-J. Diagrammatic representation of the changes that take place in the hyoidean arteries after the formation of the short circuit. The mandibular arteries are omitted in Figs. B-J. The cross connections are shown in dotted lines in Fig. F. Fig. I shows an incomplete afferent and efferent branchial system. It is complete in Fig. J. Only vessels of the first branchials are shown.

Figs. 1--11 have been reduced to $\frac{1}{2}$ the magnification given.

STUDIES IN INDIAN CEREAL SMUTS

I. Cereal Smuts and their Control by the Development of Resistant Varieties

B P PAL and B B MUNDKUR

Imperial Agricultural Research Institute, New Delhi

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(Communicated by Rao Bahadur B Viswa Nath)

THERE is hardly a cereal grown in India which is not attacked by a smut and more than one smut per cereal is the rule rather than the exception. The amount of damage which a particular smut causes depends, of course, on the extent to which environmental conditions favour the development of the fungus and varies from season to season. Several attempts have been made in the past to assess the losses occasioned by smut attack but so far as India is concerned, where accurate disease surveys have not so far been conducted, these estimates must perhaps be considered as speculative rather than authentic.

Nevertheless there is little doubt that the smuts in India cause much damage in certain areas and in certain years and there is no question that endeavours to control them are necessary and indeed have been made in the past with varying degrees of success. To devise successful control measures, however, it is necessary that one should have an accurate knowledge of the life-histories of these smuts, especially of their methods of transmission; whether they are seed-borne, soil-borne or air-borne, without which information not much progress can be made. In some cases this is, of course, already known but in others like the leaf-smut (*Entyloma Oryzae* Syd.) of rice (*Oryza sativa* L.), the grain smut (*Tolyposporium Penicillaria* Bref.) of bajra (*Pennisetum typhoides* Stapf and Hubbard) or the long smut [*Tolyposporium Ehrenbergii* (Kuehn) Pat.] of jowar (*Sorghum vulgare* Pers.) this information is not yet available. Even in the case of the bunt [*Neovossia indica* (Mitra) Mundkur] of wheat (*Triticum vulgare* Host) it is not clear whether the disease is seed- or soil-borne, for the experiments so far reported do not specifically permit any definite conclusion on this point.

In cases where it has been established beyond doubt that a smut is seed-borne, methods of treating the seed so as to control the disease have been evolved and some of them have been used with considerable success.

For example, Uppal and Malelu (1928) and Uppal and Desai (1931) in the Bombay Presidency tested the efficacy of treating *jowar* seed with several dust fungicides and observed that sulphur dust of a high degree of fineness was the best fungicidal dust for effectively controlling the grain smut [*Sphacelotheca Sorghi* (Link) Clinton]—so much so that *jowar* seed treatment has become common in the Presidency and in the neighbouring provinces where this smut occurs. For controlling both the covered smut (*Ustilago Kollerii* Wille) and the loose smut [*U. Avenae* (Pers.) Jensen] of oats, Mundkur and Khan (1934) tested the dry-spray method of applying formaldehyde to the smutty grain, which has not only given good results but the treatment has been adopted with success by several oat growers in Bihar and in the military farms elsewhere. Likewise the solar heat treatment of wheat seeds infected by loose smut [*Ustilago Tritici* (Pers.) Jensen], developed by Luthra and Sattar (1934), has also been reported to be successful and to be getting popular in the Punjab.

But seed treatments, unless they are very cheap and easy of application, like the sulphur dust treatment of *jowar* mentioned above, not only mean extra cost but involve a certain amount of danger and risk. This is especially true where substances like the organic mercury compounds and formaldehyde have to be used and in those treatments which entail the soaking of seeds in water. In such cases the solution of the smut problem in India lies in the breeding of varieties which resist the attacks of these fungi. Considerable breeding work along these lines is in progress in Europe and America.

A survey of the literature shows that so far no serious systematic endeavour has been made in India either to ascertain the degree of resistance of the cereals to their respective smuts or to evolve new varieties able to withstand these diseases. Notes based on field observations of the relative susceptibility or resistance of the cereal varieties have indeed been published from time to time but, naturally, it is not possible in such instances to determine whether the apparent resistance of the varieties was due to intrinsic resistance or whether they had merely escaped the disease.

Cereal breeders frequently accumulate, in the course of their investigations, a large number of strains obtained both by selection from existing material and by hybridisation. As a rule few of these are agriculturally important and find their way into general cultivation. The remaining varieties may, however, possess characters, such as resistance to certain diseases, which render them valuable as material for breeding purposes; before such strains are discarded it is necessary that they should be subjected

to suitable tests in order to discover those which may possess the factors for disease resistance. Such tests to discover the resistance of the strains of wheat, barley and oats bred at the Imperial Agricultural Research Institute, to the smut diseases to which they are respectively liable, are now in progress at Delhi, and it is proposed to publish the results in a series of papers, to which the present contribution forms an introduction.

Investigations on the covered smut (*U. Kollerii*) of oats were started in 1934 and those on the loose smut (*U. Tritici*) of wheat two years later. In 1937 experiments on flag smut (*Urocystis Tritici* Koern.) of wheat were included but though the work on the covered smut [*Ustilago Hordei* (Pers.) Lagerh.] of barley was started in 1935, it has made little progress because of the failure to get heavy smut attack in artificial infections.

Our experience in the course of these investigations has shown that there are certain attendant difficulties the precise nature of which must first be ascertained and suitable steps taken to overcome them before further proceeding with the work. For example, in the case of the covered smut of oats we have found that both at Delhi and Pusa the spores stored under ordinary laboratory conditions lose their viability in three or four months, contrary to the experience of workers in other countries where the spores are known to remain viable for several years. This however does not apply to the spores of the covered smut of barley, which even when stored under ordinary conditions, have remained viable for the past four years and furthermore, have not shown any falling off in their germination.

The second and the more important experience that has been gained is with regard to infection. Unless an investigator knows methods by the use of which he can bring about a high percentage of disease in a susceptible variety (see Plate XXVI), something of the order of 60 to 70 per cent. at the least, the results of his disease resistance trials are likely to be unsatisfactory and may not have any practical significance. Conditions which bring about high infection in the susceptible varieties and the proper methods of infecting the seed have therefore to be first discovered before one can undertake the development of resistant varieties. A perusal of the literature indicates that in several cases this primary precaution has not been taken with the result that the so-called resistant varieties have reacted in an inconsistent manner in subsequent years. It may be mentioned that failure to obtain encouraging results with the covered smut of barley has been due to our not yet being able to devise a method of infecting the seed which would bring about an appreciable amount of smut in the crop. But in the other cases the methods that have been developed for doing this have been highly

satisfactory. Thus in the case of the loose smut of wheat infection of the order of 100 per cent has been obtained in susceptible varieties, as for example, in Punjab C 591. In striking contrast a few varieties, including Pusa 114 and Pusa 165, have shown complete resistance (see Plate XXVII)

Finally, cognisance has to be taken of the possible existence of physiological races in the Indian smut fungi. Should further work reveal the existence of a number of such races, the breeding work will naturally be rendered more difficult. Work done in other countries on the physiological races of cereal smuts indicates that their number would not be as large as in cereal rusts.

It is the view of the authors that a breeder should be in possession of precise and accurate information regarding the degree of resistance or susceptibility of the strains of his crops to their important diseases. They feel that breeders and mycologists should whole-heartedly co-operate in such work and that the time and money spent thereupon are extremely worth while.

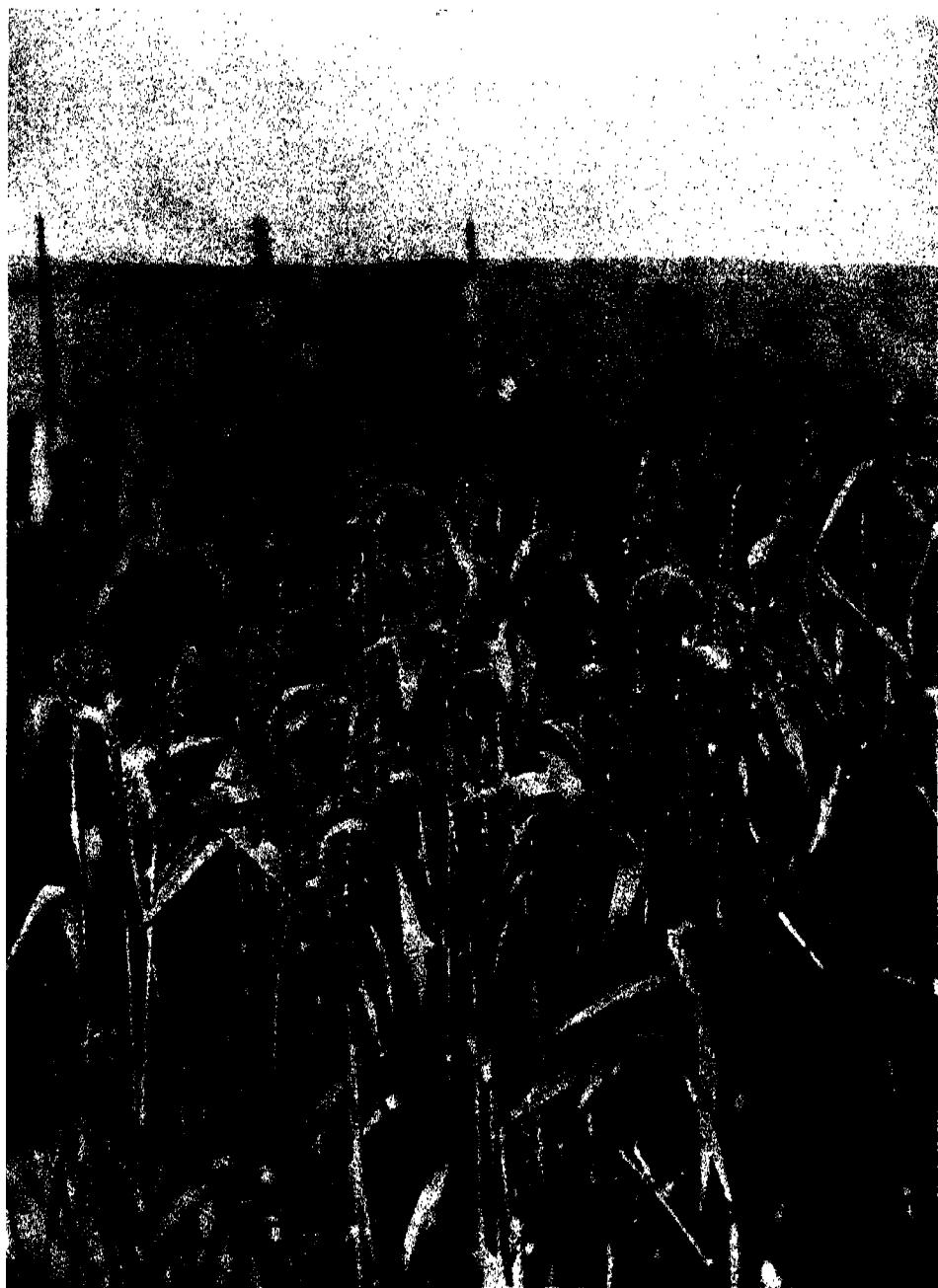
Summary

Smuts cause heavy losses to grain crops in India but before effective methods of controlling them can be devised their mode of transmission should be clearly understood. Where a smut is definitely known to be seed transmissible, methods of treating the seed have been devised but many of these methods involve a certain amount of cost and also danger. The development of disease resistant varieties helps in such cases to solve the problem.

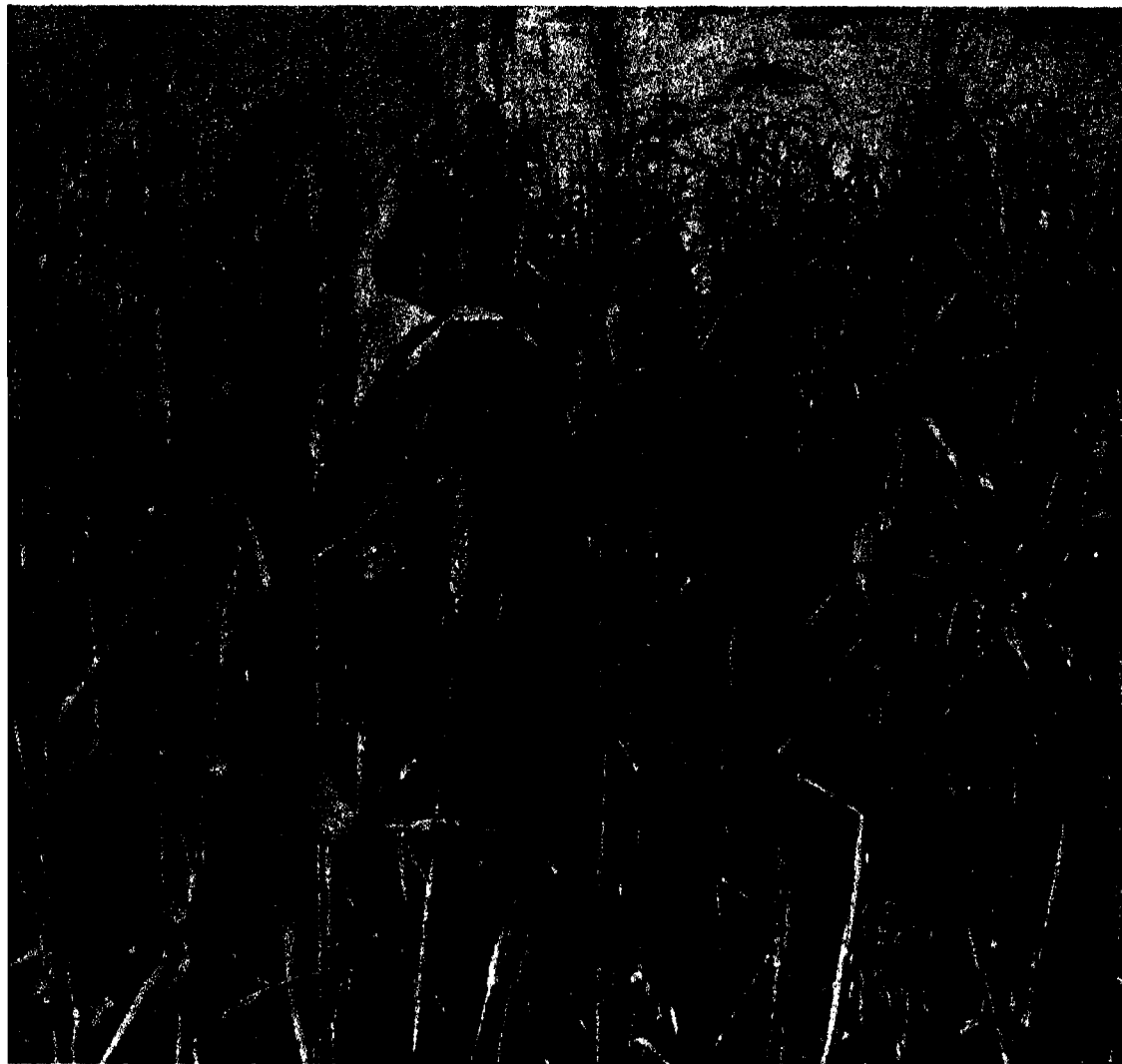
Investigations have been started in the Imperial Agricultural Research Institute at Delhi to test the relative resistance of strains of wheat, oats and barley to their respective smuts and encouraging results have been obtained which will be published in a series of papers, of which this forms the introduction.

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A variety of wheat susceptible to loose smut. Artificial infection has resulted in all ears, with the exception of one on the right, being attacked.



A highly susceptible wheat growing side by side with a highly resistant one.
Both wheats were artificially infected in the same way and at the same time.

STUDIES ON THE STRUCTURE OF THE GOLGI APPARATUS

V. The Idiosome in the Pancreas of the Toad and Its Possible Relation to the Ergastoplasm

BY M K SUBRAMANIAM, M A , D Sc

(From the Department of Zoology, University of Madras)

Received February 15, 1939

(Communicated by Prof R. Gopala Aiyar, M A , M Sc)

Introduction

SUBRAMANIAM AND GOPALA AIYAR (1936, 1937) postulated that the Golgi networks in vertebrate somatic cells should have a central chromophobic core and that while in some it may be visible, it is masked in many cases. According to this conception, the strands of the Golgi networks are comparable to plates of three-ply wood, the central sheet being the idiosome. If this is so, then, just as it is possible to separate the three sheets composing a plate of three-ply wood (Fig 1), in the case of Golgi networks with masked

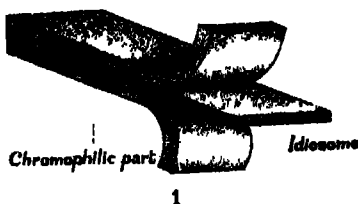


FIG 1 —A diagrammatic representation of the structure of the Golgi apparatus

idiosome also, it should be possible by some technique to demonstrate the idiosome alone. An attempt has been made in this direction in the pancreas of the toad (*Bufo melanostictus* Schneider) and the results are recorded below.

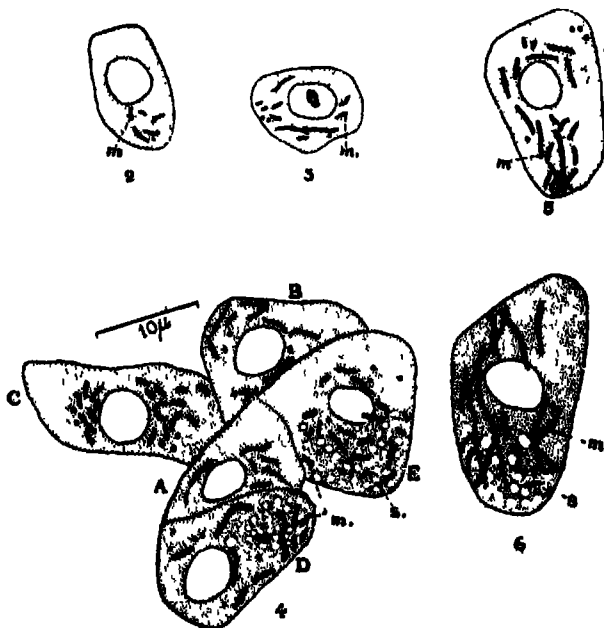
Technique

Freshly collected toads were either chloroformed or pithed and the pancreas was dissected out quickly. Various fixatives were used but in general a modification of the Mann Kopsch technique where fixation time was limited to five hours and osmication at 36° C to 62–68 hours gave beautiful pictures of the Golgi apparatus. If the pieces were thick, the mitochondria in the cells of the inner region were blackened. Thus by adjusting the thickness of the material during fixation, both the mitochondria and

the Golgi apparatus stages could be seen in the same section. Material was also fixed in Champy's fluid, Zenker, Bouin, corrosive acetic and acetic acid. Fixation in corrosive acetic or acetic acid alone gave some peculiar staining effects which will be described in detail along with the observations.

Mitochondria

In Fig 2 is shown a cell which appears to be at the beginning of secretory activity. At this stage, the mitochondria in my preparations lie on the lumen side of the cell. In Fig 3 and in Fig 4 at A and B are shown the spreading of the mitochondria around the nucleus, and in the cell at C (Fig 4) two aggregations of mitochondria occur, one on either side of the nucleus. A variation of the same distribution is given in Fig 5. It will be noticed from the figures that these mitochondrial filaments are cut transversely also. In cells in which the mitochondria alone were blackened, the secretion granules were very clear. But the pictures obtained never gave one an idea of the possibility of the secretion granules arising in relation with the mitochondrial filaments. In Fig 6 and in Fig 4 at D and E are shown the formed secretion granules. No circumstantial relation between these and the mitochondria were observed except that the secretory granules and the mitochondria lie mingled together. At the end of the secretory cycle most of the mitochondria appear to disintegrate.



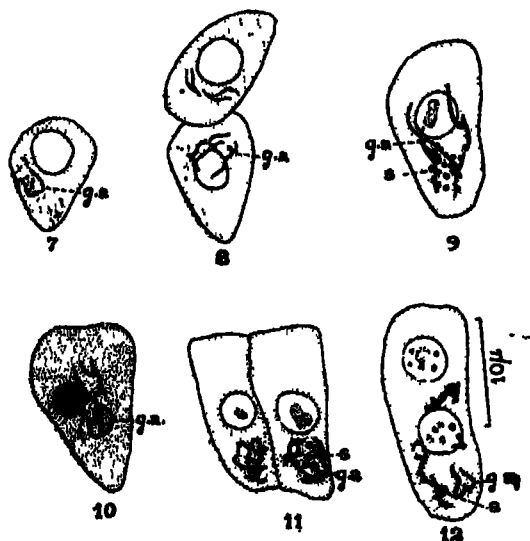
FIGS. 2, 3, 4, 5 and 6—The behaviour of the mitochondria during the secretory cycle

As the views of the recent workers on pancreas (Hirsch, 1931, 1932, Duthie, 1934) are based on staining with neutral red and janus green, I would like to point out here some of the difficulties in the acceptance of results based on staining with vital dyes. 1 Neutral red itself is a foreign substance to the cells and naturally it has got to be excreted (see Subramaniam, 1937 b). This fact necessitates a differentiation between the excreted neutral red and stained prozymogen vacuoles. (2) Koehring (1930) mentions that the action of neutral red on enzymes has a precipitating and therefore an inactivating effect. Thirty per cent of the enzyme is said to be precipitated in the vacuoles. (3) If we accept that only a non-lethal dose is injected and that the dye stains only the prozymogen, the contents of the prozymogen vacuoles should contain the precipitated compound. (4) Naturally as the dye is injected into the blood, the first appearance of these dye droplets should be in the basal region of the cell which is near the capillary. Appearance of neutral red staining bodies in the basal region of the cell therefore loses its supposed significance. (5) Even though examination carried out is that of living tissue, to all intents and purposes the contents of the prozymogen vacuoles stained by neutral red are different from those which are unstained. It is these bodies which are said to migrate to the 'Golgi zone' and get transformed into zymogen. Does it not appear that the movements of these prozymogen vacuoles stained with the dye may be due to an attempt on the part of the cell to excrete them, because they have lost the composition of prozymogen and have become actually excretory matter? In view of these considerations, it is my opinion that much reliance cannot be placed on vital staining of the prozymogen as an aid to the elucidation of the relative roles of the Golgi apparatus and the mitochondria in the production of secretion granules.

Golgi Apparatus

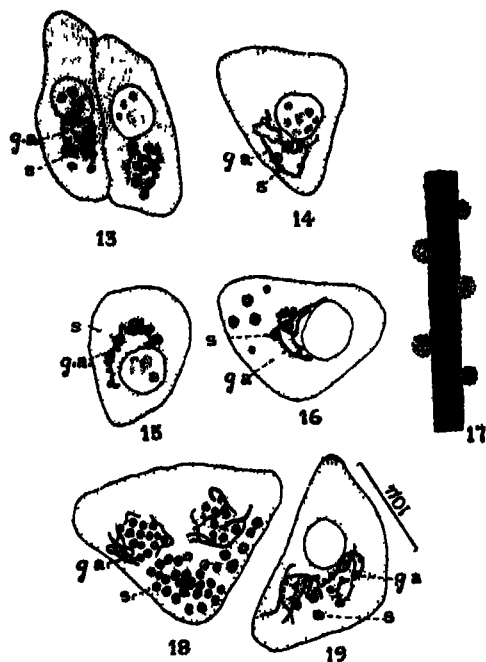
In Fig 7 is drawn the earliest Golgi apparatus. In cells at the same stage it was observed that the apparatus has either the shape of a simple network of very little complexity together with discrete rods and granules or is composed of a number of discrete rods and granules, the latter being apparently transverse sections of rods. The apparatus begins to hypertrophy—as a prelude to secretion formation—and assumes different shapes with different complexities (Fig 8). Fig 9 is of an early stage of secretory activity and the disposition of the apparatus would convince any one that it is impossible to postulate a 'Golgi zone'. It will be noticed that the Golgi strands extend beyond the nucleus into the basal region of the cytoplasm. In some cases the apparatus has been observed on one side of the nucleus extending through the cell (Fig 10). Early secretion granules were

brownish yellow and occur embedded in the strands of the Golgi apparatus (Figs 9, 11 and 12) Fig 12 seems to be a stage in the amitotic division of the pancreas cell. The apparatus occurs as a number of strands, some of which are branched, these being scattered around one of the nuclei. The



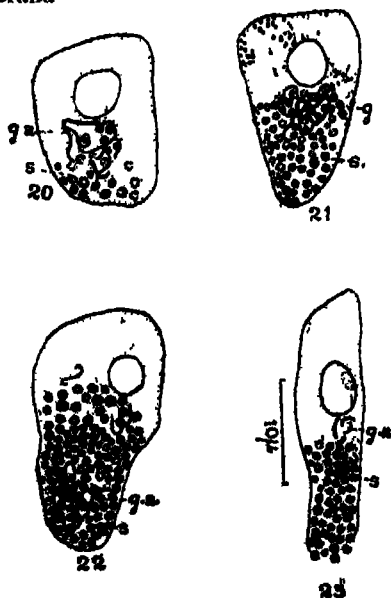
FIGS 7, 8, 9, 10, 11 and 12 —Shape and disposition of the Golgi apparatus during the early stages of the secretory cycle showing the relation of secretory granules to the Golgi strands.

size of the apparatus, which appears to be secreting zymogen suggests, as in the case of liver cells of *Rhacophorus* (Subramaniam, 1938 a), that division takes place only after a preliminary increase in size of the cell and the Golgi apparatus and the increase in complexity of the latter. The different shapes assumed by the apparatus are shown in Figs 13, 14, 15 and 16. Though all of them are almost at the same stage, they differ in the complexity of the apparatus and while in Fig 16 it lies on one side of the nucleus, it is almost basal in Fig 15. When carefully studied with the aid of a binocular eye-piece one gets the impression that the secretion granules are lodged in slight depressions on the Golgi strands (Fig 17). In Fig 18 is drawn a cell in which the apparatus occurs in two separate parts each in relation with secretion granules. These granules after they attain a certain size migrate to the basal region of the cell (Figs. 18, 19 and 20). In cells almost loaded with secretion granules (Figs 21 and 22) the network, it will be noticed, has a varied disposition in the cell and does not embrace all the granules. When the secretion granules are about to be sent into the lumen of the acini the Golgi apparatus breaks up and most of its parts seem to disintegrate.



FIGS. 13, 14, 15, 16, 18 and 19.—Different shapes, complexities and disposition of the Golgi apparatus during the secretory cycle

FIG. 17.—A diagrammatic representation of the relation of the secretory granules to a Golgi strand

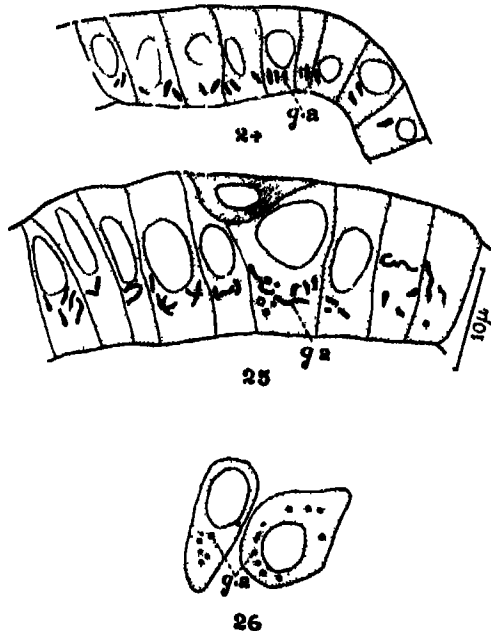


FIGS. 20, 21, 22 and 23.—The shape and disposition of the Golgi apparatus during the final stages of the secretory cycle

A few bits apparently remain as could be made out from Fig 23 where escape of the granules is by rupture of the cell membrane

In sections, well fixed cells of the ducts were also observed Fig 24 is that from a small duct and Fig 25 from a larger one The parts of the Golgi apparatus are more complex in the cells shown in Fig 25 and though no chromophobic part was seen in most cases, a few vesicles occur in some cells No actual secretory granules or vacuoles were noticed in the duct cells, but as the large duct, unlike the small one gave an impression that it contained secretion of the acinar cells, it seems probable that the varying complexity is due to the production of some product by these cells

Cells of the Islets of Langerhans contained well fixed Golgi apparatus and here also, the apparatus is constituted by discrete bodies in relation with which no chromophobic component was observed No secretory products occur in these cells and two entirely different modes of distribution of the Golgi bodies are shown in Fig 26



FIGS. 24, 25 and 26. — Discrete bodies constituting the Golgi apparatus in the duct cells (Figs 24 and 25) and islet cells (Fig 26)

It will be noticed from the descriptions and diagrams that the Golgi apparatus in the pancreas of the toad at least, shows no definite 'Golgi zone'. Neither can such a definite zone be postulated in the duct and islet

cells As in the case of the mitochondria Duthie (1934) does not trace the various changes in the Golgi apparatus during the secretory cycle He mentions that the Golgi apparatus was found in all instances to lie completely on the lumen side of the nucleus and appeared as a network embracing the zymogen granules A perusal of the papers on the pancreas (Nassonov, 1923, 1924, Bowen, 1924, 1926) indicates that the apparatus is never limited to a definite zone and that its complexity varies at different stages of the secretory activity When the secretion granules are sent out of the cells, the apparatus has been observed to break up and most of its parts either disintegrate or are extruded Thus the existence of a definite 'Golgi zone' being doubtful, Duthie's conclusions are unacceptable Proof of the existence of a 'Golgi zone' should precede the postulation of the time required to reach it from a particular portion of the cell cytoplasm, especially when the apparatus was not seen in the living cell

Bowen has described several kinds of topographical relations (1926) between the Golgi apparatus and the secretory granule and a comparison indicates that the acinar cells of the toad resemble the goblet cells of the intestine in that the secretory granules are rapidly developed in relation with the network and seem then to be gradually shifted towards the lumen by the following granules

Turning now to the intimate relation between the secretory granules and the Golgi complex, four different types were distinguished and described by me, from descriptions of various authors, in a previous paper (Subramaniam, 1938 a) In the pancreas of *Molge* and *Cryptobranchus* Bowen (1924) describes and figures the origin of secretory granules in vacuoles differentiated inside the Golgi strands and in *Triturus* also he observed a similar vacuolated appearance of the Golgi network In the cat he describes the granules as arising in association with the outside of the strands of the Golgi network Golgi apparatus in the toad resembles that of the cat for, it does not at any time show a vacuolation in the strands and the zymogen granules arise in relation with the outside of the strands and not actually embedded in the substance of the strands

In passing I would like to mention here the fact that not all vertebrate cells can be postulated to contain a Golgi network (see Subramaniam, 1939) In the toad it would have been noticed, that while a network occurs in acinous cells, the duct and islet cells contain only discrete bodies Both however agree in that no chromophobic component—except in some vesicles in duct cells—have been observed in relation with the apparatus Bensely (1911) has shown by vital staining of islet and duct cells that there is epithelial continuity between the ducts, islets and the acini Does the

similarity in the absence of a network in the duct and islet cells have any significance ?

Idiosome

In Parts I (Subramaniam and Ganapati, 1938), II, III and IV (Subramaniam, 1938 *a*, *b* and *c*) of these Studies, it was demonstrated that the idiosome has no relation to the centrosphere. Further it was shown in the liver cells of *Rhacophorus* that the Golgi apparatus actually differentiates an idiosome during the active synthesis of bile components. In the pancreas cells of the toad no chromophobic part occurs in relation with the outside of the Golgi strands, rods and granules. Hence an attempt was made to unmask the idiosome by technique already employed by other workers to demonstrate it in germ cells (Bowen, 1928). An effort was also made to demonstrate the centrosome and idiosome side by side. It was argued that if the chromophobic part appears in a masked condition according to our postulate (Subramaniam and Gopala Aiyar, 1936, 1937), modifications of the methods given by Bowen should prove successful. The method of Prenant (1902, 1905, Laugeron, 1925) has been found successful by several workers (Champy, 1923, Poisson, 1927) and as this method was originally developed for the detection of mucin which is of a proteid nature, it logically follows that the idiosome should also be of a proteid nature.

Cowdry (1925) mentions, that when preparations of the Golgi apparatus in the acinous cells of the pancreas were bleached and restrained with iron hæmatoxylin, clear canals were seen in the place of the blackened networks. The first experiment tried was in this direction. But here several factors affect the demonstration of the idiosome. If the material is bleached quickly only clear canals are left but this is not universal for, in actual experiments, I have noticed the cytoplasm bounding the strands of the apparatus getting jagged and giving rise to indistinct pictures. Slow bleaching extending to several days and later staining gave some pictures in which certain strands were seen in the position previously occupied by the apparatus, but these had a fibrillar appearance. Therefore an attempt to demonstrate the chromophobic core in osmicated material had to be given up due to the suspicion that the idiosomic material is dislocated and distorted from its position when treated with bleaching agents. Moreover, osmicated material even when bleached does not stain well.

This experiment gave me an idea that the removal of the chromophilic part should be done before sectioning. With this end in view material was fixed in Bouin's fluid, stained with iron hæmatoxylin, followed by erythrosin and light green. The order of staining was also reversed erythrosin being used first followed by iron hæmatoxylin and light green. According to

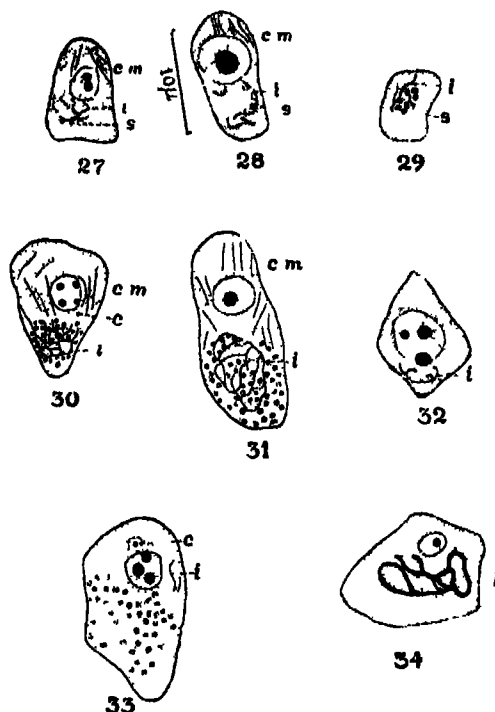
Bowen the idiosomic substance should be coloured green and he mentions that staining with light green is the most difficult point. As could be expected, the chromatin which is almost invisible in Mann Kospch is stained in Bouin preparations. The secretory granules stain green and the idiosome stains from dark rose to green. If merely stained with hæmatoxylin it is blue or light blue and when followed by the eosin dye it gives a mixed colouration due to the superimposition of the eosin dye on that of the hæmatoxylin. When slides are differentiated till chromatin alone is stained and followed by erythrosin, then what appears to be the chromophobic portion of the Golgi apparatus and mitochondria stain with erythrosin and if followed by light green these structures are stained green. Personally I would prefer preparations in which the chromophobic part does not stain with light green, for in such sections chromatin, idiosome and zymogen are stained in different colours. For the purpose of photomicrography it is preferable to stain with iron hæmatoxylin alone. Before describing the stages and pictures seen I would like to mention here the conception of the structure of the mitochondria. The filaments observed in the pancreas of the toad have no visible chromophobic areas. Similar filaments have been noticed by Bowen (1922) to differentiate a chromophobic area during nebkern formation in insect spermatogenesis. He conceived these chromophobic areas as forming a core to the filaments. The cores of the mitochondrial filaments were also stained in a manner similar to the idiosome in the technique described above. In Fig. 27 is a cell in which some thin strands are seen on the sides of the nucleus and an extremely simple net-like structure below the nucleus. The strands of the latter structure had in relation with it a few secretory granules stained green. A slightly later stage is given in Fig. 28 where it was difficult to determine whether the free strand just below the nucleus was the core of a mitochondrial or Golgi filament. The area near the strands of what apparently constituted the idiosome of the Golgi network are stained in a lighter shade of green than the zymogen granules (Fig. 29). Figs. 30 and 31 are cells in which secretory granule formation is well advanced. In these, as in previous figures, the secretion granules had a dehydrated appearance. A comparison of Figs. 30 and 31 with those of cells at a similar stage (Figs. 4, 6, 19, 21 and 22) in which the Golgi and mitochondria are observed will indicate how the two pictures are superimposable. In Part I of these Studies (Subramaniam and Ganapati, 1938), I traced the history of the term 'archoplasm' and pointed out that in *Lecudina* the idiosome is a separate entity having no relation to the sphere substance. In some acinar cells (Fig. 30) the centriole and centrosphere are seen along with the chromophobic parts of the Golgi and mitochondria and it will at once become

apparent how the sphere substance or 'archoplasm' can have nothing to do with the chromophobic part of the Golgi

Corrosive acetic fixations were made chiefly to demonstrate the centriole according to Heidenhain's staining directions, and Figs 32 and 33 are taken from such preparations. A peculiarity of these preparations is that the idiosome alone was stained by iron hæmatoxylin. It appears to me that the selective staining of the idiosome alone may have given rise to structures called 'Nebenkerns' by earlier workers. The cell in Fig 32 is at a very early stage in the secretory cycle, and the one in Fig 33 is about to extrude the zymogen and in which a greater portion of the idiosome seems to have disintegrated (compare with Fig 23). Cowdry (1934) mentions that a centriole when present in such polarised cells as the acinar cells, invariably occupies a position between the nucleus and lumen in the distal protoplasm. In the cell indicated in Fig 33, however, which is loaded with secretion it occupies a position in the basal region of the cytoplasm near the nucleus.

As the idiosome seems to be of a proteid nature—suggested by its presence in fixatives containing picric and acetic acids or corrosive sublimate—it was thought that it should be possible to make it swell and thus improve its clarity. In "Microtomists Vade Mecum" it is mentioned that Flemming made a special investigation of the action of acetic acid on nuclei and found that strengths of 5% and more bring out the nuclein structure clearly at first, but after a time cause them to swell and become pale. It has also been admitted that the strong acid is a valuable fixative for, it kills with the utmost rapidity, and leaves it fixed in a state of extension. The chromatin and chromophobic parts of the Golgi and mitochondria are seen side by side in Boun fixed material and it was argued that if acetic acid swells the chromatin it should swell the chromophobic component also. With this end in view the proportion and strength of the acetic acid in picro-formal-acetic and corrosive acetic mixtures were increased and it was found that an addition of 10 cc of glacial acetic to every 100 cc of Boun's fluid and 15 cc to every 100 cc of corrosive sublimate in normal saline gave the best results. The time of fixation has necessarily to be short. In the former preparations the chromophobic component of the Golgi and mitochondria and in the latter the idiosome alone were slightly swollen along with the chromatin. As I am mainly concerned in this paper with the idiosome and as acetic acid or corrosive acetic gave good pictures of this structure alone, material was fixed in 5% acetic acid and the idiosome was allowed to swell. The picture obtained is shown in Fig 34. Careful differentiation of the iron hæmatoxylin is necessary as otherwise the whole area occupied by the idiosome is either black or blue according to the length of treatment with the

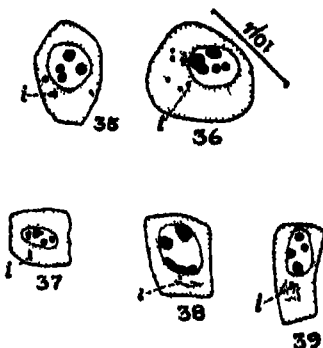
stain Concurrent with the swelling some slight changes were also seen In Boun slides stained according to Prenant's technique the cytoplasm in between and near the strands were never stained in a colour similar to that



FIGS 27, 28, 29, 30, 31, 32, 33 and 34 —Illustrations 27, 28, 29, 30 and 31 are from material treated according to Prenant's technique and show the chromophobic portions of the mitochondria and the Golgi apparatus Figs 32 and 33 are from Corrosive acetic preparations where the idiosome alone is seen The swollen idiosome in acetic acid preparations is shown in Fig 34 The centriole may be seen in Figs 30 and 33

of the idiosome But in material fixed in 5% acetic acid while the strands of the idiosome were blue, the cytoplasm within the strands was tinged lightly and differed in colouration from that of the other parts of the cell If, however, the iron hæmatoxylin is followed by Bordeaux R such a differential staining of the cytoplasm disappears

It was pointed out while giving an account of the Golgi apparatus, that in the duct and islet cells the apparatus is constituted by discrete bodies No chromophobic component for these bodies were observed and if one has to accept the structures seen in acinar cells as the idiosome and the chromophobic part of the mitochondria, in the duct and islet cells also the chromophobic component should be seen Certain pictures were obtained in material



Figs. 35, 36, 37, 38 and 39 —Swollen idiosomic component of the Golgi apparatus of the islet cells (Figs 35 and 36) and duct cells (Figs 37, 38 and 39) in acetic acid preparations

treated according to Prenant's technique but these were not very satisfactory. As the difficulty encountered was due to the extreme small size or tenuity, it was thought that slight swelling in acetic acid should improve the clarity. Thus supposition was justified and in Figs 35 and 36 are drawn two islet cells and in Figs 37, 38 and 39 three duct cells. A comparison of these with the pictures of the Golgi apparatus (Figs 24, 25 and 26) will, I think, justify the conclusion that what are seen in acetic acid preparations are after all the idiosome.

Is the Ergastoplasm the Chromophobic Part of the Golgi Apparatus and Mitochondria?

From entirely theoretical deductions I put in a query (Subramaniam, 1937 c) whether it is not possible that what is described under the term 'Ergastoplasm' may after all be the chromophobic component of the Golgi and mitochondria. A brief review of what is the 'Ergastoplasm' was given and the objections and resemblances of the Ergastoplasm to the idiosome and the chromophobic part of the mitochondria were discussed. Here I shall limit myself to a discussion of the objections against the Ergastoplasm being the chromophobic component. Some of the important considerations raised by Bensley (1911) are (1) Filaments of Solger occur only in gland cells and were not seen in epithelial cells, (2) the Ergastoplasmic fibrillae were not seen in living material, (3) that the mitochondria are coarse and bacillus-like while the filaments of Solger are fine and often form an intricate skein. It will be seen from the descriptions based on acetic acid, or Bouin or corrosive acetic preparations of the pancreas that structures comparable to the Ergastoplasm are observed in epithelial cells as well as gland cells and because these form a core to the mitochondria—and also the Golgi apparatus—

one can possibly see only the mitochondria in vitally stained cells and not the core and mitochondria separately. The supposition that the mitochondria are separate from Solger's filaments seem to have pervaded the whole of the recent studies and reviews of the Ergastoplasm and in view of this confusion we find Cowdry (1934) stating that a basophilic material containing iron appears as a homogeneous material in the ground substance of the proximal cytoplasm of acinar cells. He says that when poorly fixed this substance appears "as strands of material (the basal filaments of Solger) between the spaces occupied by the mitochondria" (p 265). His suggestion is that the impossibility of its detection in living cells is due to its amorphous condition. Cowdry rightly hints that in the suggestion mentioned above one is faced with premature generalizations. No worker to my knowledge has shown clearly the filaments of Solger—having no possible relation to the idiosome—occupying the spaces between the mitochondria and both being seen in the same cell.

Having stressed the possibility of the central chromophobic core of the mitochondrial filaments being partly the structures known as Ergastoplasm I shall now pass on to the idiosome being also responsible for the production of Solger's filaments, especially the skein observed by many workers. Unfortunately here also the question of invisibility in living tissue of the Golgi apparatus itself comes in. As emphasized by me in a previous paper (Subramaniam, 1937 *a*) criteria employed to accept chromatin as a definite entity are not employed to the acceptance of Golgi apparatus as a definite living cell inclusion. A perusal of the Figs 1-11 in Covell's paper (1928) and Fig 6 in that of Duthie (1934) will show that during vital studies of the pancreas acinous cells no structural details were observed inside the nucleus. Yet, this has never formed an objection to a consideration of the chromatin as a definite nuclear structure whereas a similar invisibility in the case of the Golgi apparatus has been considered to be a bar to its acceptance as a definite living cell inclusion. I showed that criticisms levelled against the existence of the Golgi apparatus if applied to chromatin prove that chromatin may also be an artifact. Just as the invisibility of the chromatin is accounted for by the identity of its refractive index with that of the nuclear substance in which it lies, I believe a similar condition explains also the invisibility of the Golgi apparatus. Thus while the Golgi apparatus itself is invisible in many cases it is too much to expect a demonstration of its chromophobic core in vital material. In my paper on the theoretical aspect of this question I hinted that the difficulty in exactly superimposing pictures of Solger's filaments on that of mitochondria and Golgi apparatus may be due to the production of currents in the cytoplasm

due to removal from the tissues of fats and lipoids during dehydration, etc. My results on the pancreas suggest that such distortion is only slight and pictures obtained are superimposable.

After the demonstration of the idiosome and the chromophobic part of the mitochondria in the acinar cells and the idiosome in the duct and islet cells where the apparatus has not the shape of a network at all, I believe it will be admitted that what were in many cases described by innumerable authors under the term *ergastoplasm* were not artifacts at all but were really the chromophobic components of the Golgi apparatus and mitochondria.

Bowen wrote in 1928 "The attempt to extend this conception of the duplex constitution of the Golgi apparatus to all kinds of cells has not yet resulted in a satisfactory conclusion (Bowen, 1926 a), and the whole matter is in much too uncertain a phase to warrant any decided statement one way or the other. If the presence of this chromophobic material could be proved in all cells, our understanding of the whole Golgi apparatus problem would be at once greatly advanced" (p 104). Theoretical evidence extending the duplex structure to the vertebrate network was presented in three previous papers (Subramaniam and Gopala Aiyar, 1936, 1937, Subramaniam, 1937 c) and in the present investigation an attempt has been made to unmask the postulated chromophobic core.

The demonstration of one component at least of the Golgi apparatus along with chromatin in fixatives ordinarily employed in histological studies has a wider significance. While showing that the apparatus has a material existence in the cell and that it has an identical duplex structure in vertebrate somatic cells as well it goes to prove that the idiosome has nothing to do with the centrosphere or archoplasm as supposed by many workers. This demonstration of the idiosome in Bouin, corrosive acetic and acetic acid substantiates our (Subramaniam and Gopala Aiyar, 1936, 1937) supposition that it forms a core to the strands composing the Golgi network in vertebrate somatic cells.

Summary and Conclusions

The Golgi apparatus and mitochondria were studied in acinar, duct and islet cells of the pancreas. It is shown that there is no 'Golgi zone' and that the secretion granules arise in relation with the Golgi apparatus. The view of Duthie that the prozymogen granules arise in relation with the mitochondria is discussed.

What appears to be the chromophobic components of the Golgi apparatus and mitochondria are demonstrated in acinar, duct and islet cells. It is suggested that what are described by earlier workers as "*Ergastoplasm*" and "*Solger's filaments*" are after all in many cases the idiosome and the chromophobic component of the mitochondria. The presence of such

structures in all types of cells in the pancreas where they have different shapes and which are superimposable on pictures of the Golgi apparatus obtained in the same cells suggests that the descriptions of many of the earlier workers are correct and that these are not artifacts. The suggestion of Subramaniam and Gopala Aiyar that the idiosome may form a core to the Golgi apparatus is substantiated.

Acknowledgment

I would like to express here my indebtedness to Prof R Gopala Aiyar, Director, University Zoological Research Laboratory, for his suggestions, criticism and encouragement.

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KEY TO LETTERING IN FIGS.

c., Centriole; c.m., Chromophobic component of the mitochondria, g.s., Golgi apparatus; i., Idiosome; m., Mitochondria; n., Nucleus; s., Secretion granules.

LIFE-HISTORY OF *STEPHENSONIELLA* *BREVIPEDUNCULATA* KASH.

BY P N MEHRA AND H L MEHRA

(From Kashyap Research Laboratory, Panjab University, Lahore)

Received February 4, 1939

[Communicated by Dr H Chaudhuri, D Sc (Lond), Ph D , D I C]

Stephensoniella brevipedunculata—the monotypic genus, was first discovered by Kashyap (1914) The species has a very restricted distribution occurring only in the outer ranges of the N W Himalayas where it is very common at Mussoorie, Simla, Kulu, etc , at an altitude of 6,000–7,000 ft The plants are dioecious They grow either singly hidden among the grass and mosses or in large almost pure patches of male or female plants on bare rocks in more or less shady places

The material was collected from Mussoorie in 1932 and 1935 and from Rahla in Kulu in 1933 Bouin's fluid Allen's modification P F B,¹⁵ Nawaschin's fluid and Flemming's solution for Gasteria were tried as fixatives out of which the first gave the best results

Sections were cut 6–10 μ thick Staining was mostly done in Haidenhain's iron-alum hæmatoxylin Delafield's hæmatoxylin was also used in some cases

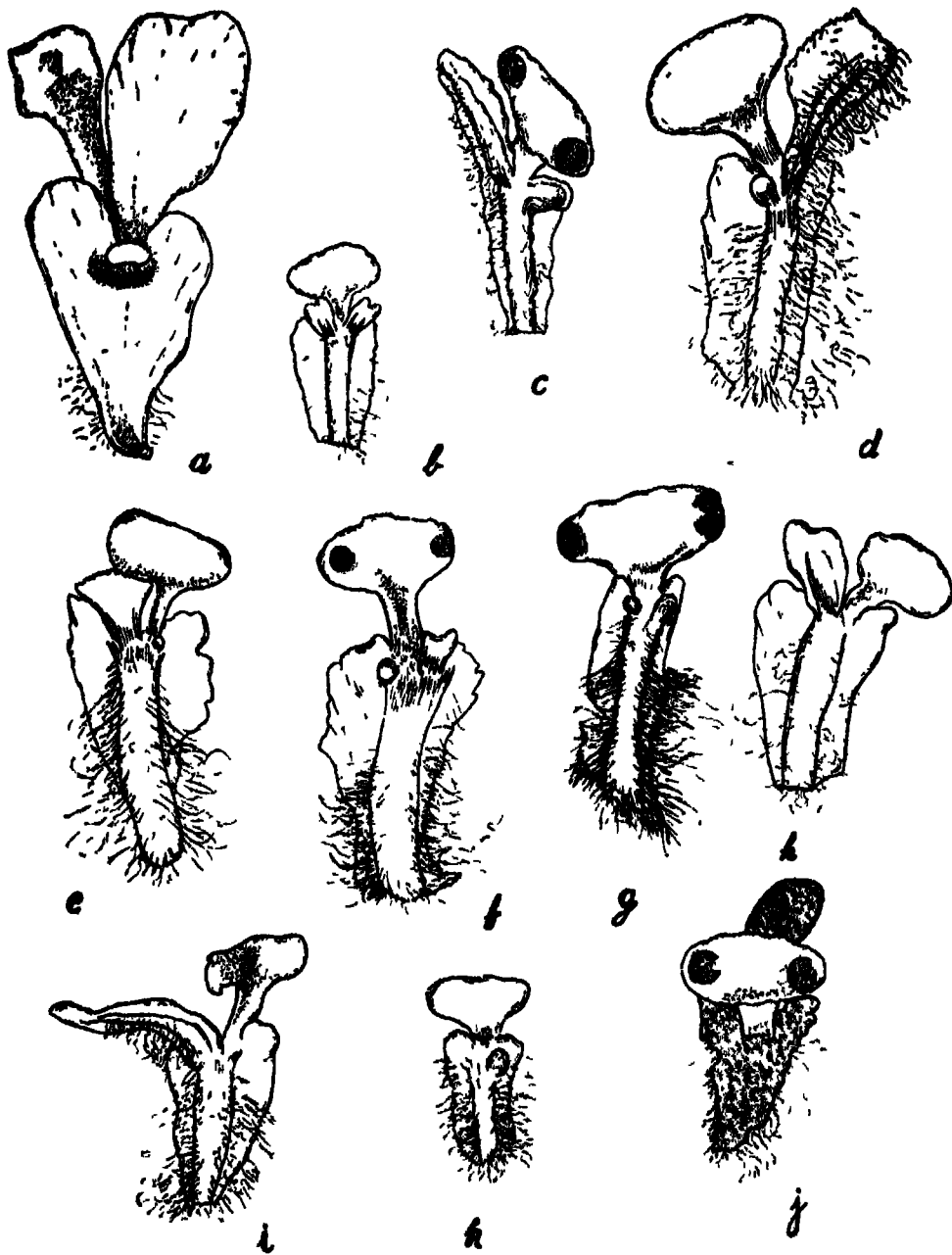
Thallus Morphology

The species is strictly dioecious No monoecious individual has either been observed or recorded

The plants are very spongy in texture and light-green in colour Each consists of a dorsiventral thallus which may be simple or once or twice dichotomously branched The lobes are obcordate There is a thick midrib projecting ventrally from which the rhizoids spring which attach the plant closely to the soil The plants usually possess a thick base indicating that they have been formed from the tubers of the last year

The male plants can easily be recognised when the anthegidia ripen by the reddish brown streaks on the dorsal surface which are the male receptacles

Rhizoids are of two types, the smooth-walled and the tuberculate The latter possess peg-like thickenings on their inner walls Both occur intermixed and spring from the lower surface of the midrib each as an outgrowth from a single superficial cell

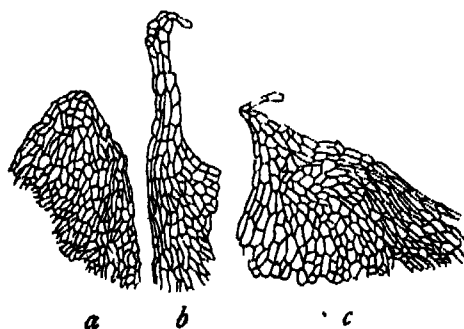


TEXT-FIG. 1

Female plants. $\times 6$

- a*.—Two well-developed sub-apical lobes formed after the formation of female receptacle which is young Dorsal view
- b* —Sub-apical lobes rather poorly developed Ventral view
- c* —The sub-apical lobes unequal in size Ventral view
- d, e, f*.—One sub-apical lobe well developed, the other in the form of a tuber
The point of insertion of these on the midrib is fairly apart from one another
Ventral view
- g* —Both the sub-apical lobes in the form of tubers and fairly apart from one another Ventral view
- h, i* —Only one sub-apical lobe developed, the other completely suppressed
Ventral view
- j* —Another plant of the same type as *h, i* in dorsal view, giving a false appearance as if continuation of the apical region after forming the female receptacle has occurred
- k* —Only one sub apical lobe and that too in the form of a tuber

There are two rows of scales on the undersurface of the thallus, one on either side of the midrib from where they arise They reach about half-way between the midrib and the margin of the thallus The mature scales are large and purple in the posterior half of the thallus Kashyap (1914) describes the scales to be hyaline which is not the case They have a smooth margin and possess either no appendage or commonly only one appendage at or near the apex 2-4 cells long (Text-Figs 2 *a, c*) Sometimes the apical region of the scale may be drawn out (Text-Fig 2 *b*) Kalsyap states (1914) that a few filamentous appendages may be present on a scale at the apex or along the margin There are no mucilage cells but in the posterior region of the thallus the cells in the centre of the scales contain a purple colouring



TEXT-FIG 2

- a*.—Scale without appendage × 33
- b*.—Scale with the apical region drawn out bearing a terminal two-celled appendage × 33
- c*.—Scale with 4-celled appendage. On the right margin the cells have been newly formed and are narrow and hyaline. × 33

matter which imparts the characteristic colour to the scales. The posterior margins are usually hyaline with narrow elongated cells possessing thin walls. It appears that new cells are added along this margin for sometime even after the scales mature. The young scales are green in colour.

The anterior margin of a scale overlaps the posterior margin of the scale just in front of it. Near the apex, the anterior regions of the scales on both the sides curve inwards and upwards thus closely protecting the growing point.

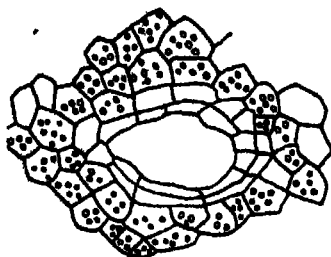
Internal Structure

The structure of the thallus is not very complex. There is a single layer of thin-walled cells forming the epidermis. These cells generally rupture in the living condition, and are perforated by simple pores which communicate below with a zone of air-chambers. The large pores give the plant its spongy appearance. Each pore is surrounded by one or two rings of 6-10 more or less hyaline cells (Text-Fig. 3). The cells of the inner ring when there are two, are usually collapsed and form a thin membrane which projects into the interior of the pore. In surface view the pores appear circular, ovoid or more or less elliptically elongated.

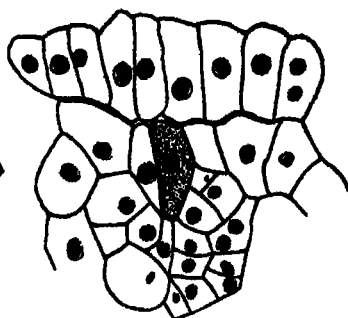
The chambers are arranged in a single layer and are obliquely directed forward. Because of this they overlap and consequently in thin microtomic sections give a false appearance of being arranged in several layers, (Pl. XXVIII, Fig. 1). They are large and well-developed and are devoid of assimilating filaments. In this respect the genus resembles *Astichisoniella*, *Athalamia*, *Sauchia*, *Cyathodium*, *Cryptomitrium*, etc.

Each chamber opens by a pore. Frequently two pores of the adjacent chambers fuse forming a large pore which communicates with both the chambers. In such cases the partition wall of the two chambers does not reach the epidermal surface. Kashyap (1914) finds that a pore may sometimes communicate with four chambers.

The ventral tissue is formed of closely-packed parenchyma. There are no sclerotic cells of the type stated by Goebel (quoted by Campbell, 1918) for *Preissia commutata* in the midrib, nor are there any reticulate thickenings on their walls which are particularly conspicuous in *Marchantia*. This tissue is 12-14 layers of cells thick in the region of the midrib. The cells just below the air-chambers are large and contain numerous chloroplasts. Towards the lower surface there are 2-3 layers of smaller cells. Mucilage cells which assume a dark grey colour with iron-alum hæmatoxylin are found scattered in this tissue. Pl. XXVIII, Fig. 3 shows a photograph of the ventral tissue with mucilage cells. Cavers (1904) reports that in *Conecephalum*



TEXT-FIG 3

Text-Fig 3—A pore as seen from surface $\times 107$ 

TEXT-FIG 4

Text-Fig 4—Apical cell in L. 4 of the thallus $\times 667$

the mucilage cells are not isolated but may form rows of cells which unite to form mucilage ducts. Starr (1916) observed mucilage cells singly scattered in *Plagiochasma* (= *Aytoma*) and Haupt (1926) observed the same thing in *Preissia quadrata*. In *Reboulia hemisphaerica* oil cells occur in this tissue (Haupt, 1921).

No mycorrhiza is present in the ventral tissue.

An interesting feature is the common occurrence of two types of algal colonies in the air-chambers. In the first type the colony is composed of unicellular more or less globular cells variable in size. Text-Fig 5 *a* shows a few such colonies in a chamber and in Text-Fig 5 *b* one such is shown magni-



TEXT-FIG 5

a.—A chamber containing a few algal colonies with globular cells $\times 58$

b.—One of the above colonies magnified $\times 300$

c.—Second type of algal colony, *Nostoc*-like, which resides in air-chambers $\times 317$

fy. The second type is composed of a number of beaded filaments of different sizes much like that of *Nostoc* (Text-Fig 5 *c*). These colonies seem to enter the air chambers through the large pores and appear to have a merely

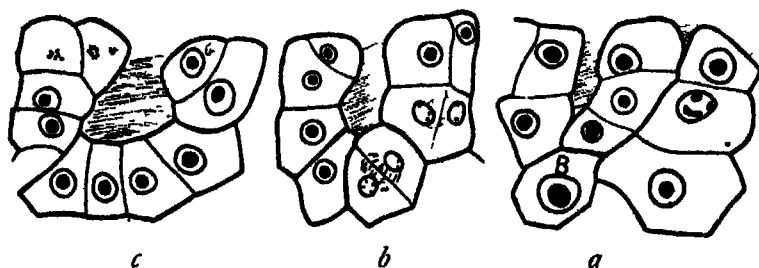
incidental association with the plant. These are often met with between the scales and the ventral region of the thallus and sometimes are lodged in cavities scooped out of the ventral tissue of the thallus.

Growing Point

The growth of the thallus occurs by a four-sided apical cell which cuts off segments dorsally, ventrally and on the two lateral sides. In a vertical section in Text-Fig. 4 it appears two-sided and the segments alternately on the dorsal and ventral sides are clearly observed.

Origin of Air-Chambers

The origin of air-chambers in *Stephensoniella* takes place schizogenously in the manner described by Barnes and Land (1907). Cleavage occurs along the vertical walls from above downwards, in the newly formed dorsal segments behind the apical cell, until it reaches the cell formed of the ventral segment. This cell is marked 'B' in the Text-Fig. 6 *a*. In the meantime the dorsal segments have divided by transverse walls usually giving rise to



TEXT-FIG. 6

Showing different stages in the origin of air-chambers $\times 465$

2 or 3 cells. The cell of the ventral segment divides a number of times by vertical walls (Text-Figs. 6 *b*, *c*) forming a board floor with the result that the cavity also broadens particularly at the base and becomes more or less flask-shaped. This division continues for sometime. In the meantime cells are cut off from the uppermost cells of the dorsal segments towards the inside *u u'*. These cells by their further growth and division towards the centre, form a roof of the chamber leaving an opening or pore in the middle.

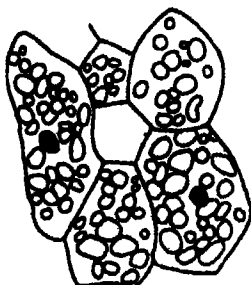
Vegetative Reproduction

Sometimes vegetative reproduction takes place by thick adventitious ventral shoots produced by the plant. As the main thallus decays, these get detached and by further growth produce new plants.

Mostly the vegetative reproduction, which incidentally is also the method of perennation in the species, is by the formation of apical tubers. They are formed at the end of the vegetative season (during the latter half of September) from every branch that lives upto that period.

Each tuber is more or less cylindrical in form. It is closely surrounded by overlapping scales and burrows into the soil to which it is attached by both the smooth-walled and tuberculate rhizoids.

A section of the tuber shows that the outer wall is formed of one, two or three layers of cells without contents and with walls corky in nature as shown by microchemical tests. Some cells of the outer layer on the lower side of the tuber are produced into long smooth-walled and tuberculate rhizoids (Pl XXVIII, Fig 2). Within the protective covering in the lower region of the tuber is compact thin-walled parenchyma comparable to the ventral tissue of the thallus but is much better developed here. The upper and middle region of this is densely stored with starch grains of different sizes as shown by a few cells drawn from this region (Text-Fig 7). In the lower region the



TEXT-FIG 7

Starch loaded cells from the ventral compact region of the tuber

The central is the mucilage cell × 533

cells are not so densely packed and also the starch grains are smaller in size. Starch is also accumulated in the lower cells forming the wall of the chambers. Mucilage cells like those present in the thallus are also met with. One such cell enclosed by starch-bearing cells is shown in Text-Fig 7. The nature of food material in the tubers of *Stephensoniella* differs from that in tubers of *Anthoceros tuberosus* where it is of the nature of oil drops and aleurone grains (Ashworth, 1896).

In the upper surface of the tuber are present narrow air-chambers compactly arranged and obliquely directed forward, so that they overlap and in thin microtomic sections give the deceptive appearance of being in many layers.

The tuber thus is only a very much condensed thallus with both scales and rhizoids, the latter of two types as in the thallus, and densely stored with starch to tide over unfavourable winter conditions. The observation of Kashyap (1914) that there are no air-chambers in the tubers of *Stephensoniella* is not supported by the present investigation.

An interesting feature is that the tubers developed from the male plants bear on their upper surface and sunk in the tissue a number of antheridia developed in an acropetal succession from the apex backwards. The antheridia are formed in antheridial chambers (Pt XXVIII, Fig 2). These become dormant at different stages of their development on the arrival of unfavourable winter conditions and complete their development in the next season. Thus these antheridia take two seasons for their complete development. This incidentally explains the difficulty in obtaining early stages of antheridium development in plants fixed even quite early in the vegetative season.

The antheridia are never found on tubers developed from female or sterile plants. To the knowledge of the writers antheridia have never so far been reported from the tubers of any Marchantiales.

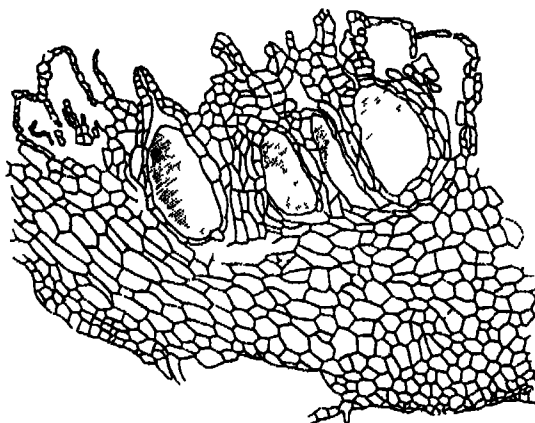
The formation of the tuber takes place by the segmentation of the apical cell of the thallus or its lobe. The apical cell survives in the tuber protected over by scales while the rest of the thallus dies. On the return of favourable conditions it renews its activity and forms a new individual. Being formed from a tuber the posterior region of the new plant is thick and bulbous.

Sex Organs

The plants are strictly dioecious. The male plants occur in much smaller numbers in nature than the females. Also the patches of male plants occur at fairly large distances from the female patches. Again most of the antheridia formed on the tuber in the previous year mature early in the next vegetative season when the archegonia are not yet fully formed. These factors account for poor fertilization and only a few female plants produce ripe sporogonia. Besides the male and female plants a fairly large number of individuals are sterile in nature.

Male Receptacle—The antheridia occur in a broad median dorsal groove in two or three alternating rows forming a definite receptacle of the simplest type known in the *Compositae* of the *Marchantiaceae*. Other allied genera *Exormotheca* and *Aitchisoniella* also possess similar simplest type of male receptacle. In *Stephensoniella* the receptacle sometimes branches along with the bifurcation of the thallus. Frequently the two branches after growing for some distance may again bend inwards and fuse leaving an island of vegetative region enclosed within the receptacle as described by Kashyap (1929).

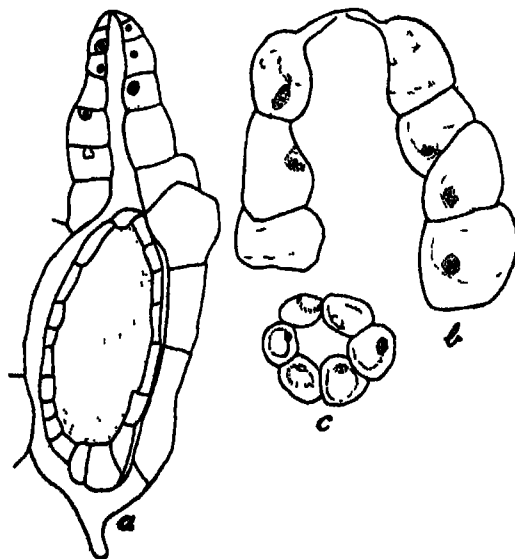
The antheridia grow in an acropetal succession from the front backwards. Each antheridium is formed within an antheridial chamber (Text-Fig 8) which opens outside by a conspicuous papilla composed of 4-8 tiers of cells



TEXT-FIG 8

A part of the male receptacle in vertical longitudinal section $\times 58$

(Text-Fig 9 a) In each tier there are six cells as shown in a transverse section of the papilla (Text-Fig 9 c). The uppermost or opercular cells of a



TEXT-FIG 9

a.—An antheridium in an antheridial chamber. The papilla closing the cavity. $\times 300$

b.—A papilla in V.S. showing beak-like processes given from the uppermost cells. They help to close the antheridial chamber $\times 300$

c.—A T.S. of a papilla showing 6 cells. $\times 300$

papilla are either conical in form (Text-Fig. 9 a) or flat with beak-like processes (Text-Fig. 9 b). In either case the beak-like processes or the conical cells meet at their apices and close the antheridial chamber when the antheridia are young. On their maturity the papillae open, assume a conspicuous dark red colour which imparts a characteristic tint to the male plants which can then be easily recognised. There are no air-chambers between the antheridia as are met with in some *Marchantiaceae* (Text-Fig. 8).

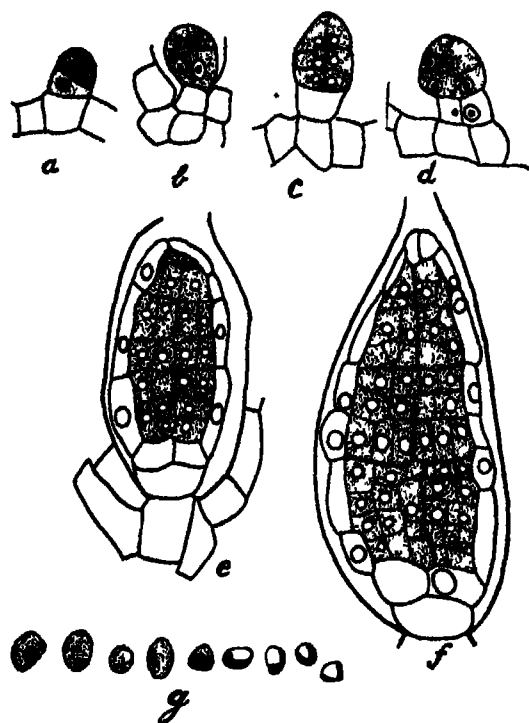
Antheridium Development—A superficial cell is cut off from the upper surface of the thallus in the median dorsal groove in the form of a papilla. The adjacent tissue gradually grows around it and consequently it comes to lie in a pit. It divides by a transverse wall, which sometimes is oblique, into a lower stalk cell and an upper antheridium initial (Text-Fig. 10 a). Then a transverse or a slightly oblique wall is laid down in this initial followed by another similar one giving rise to three superimposed cells. In each of the three cells two vertical walls at right angles to each other are formed (Text-Fig. 10 c). Thus three tiers with four cells in each tier are formed. Periclinal walls are next laid down in these cells forming a layer of outer peripheral wall cells as distinguished from the inner primary spermatogenous tissue.

Occasionally vertical walls are laid down immediately after the first transverse division of the antheridium initial (Text-Fig. 10 b).

Ahmed (1938) in *Aitchisoniella* and Haupt in *Reboulia* (1921) and *Preissia quadrata* (1926) and Strasburger in *Marchantia* (quoted by Haupt, 1926) find that a row of four cells are formed before vertical walls are laid. In *Fimbraria* investigated by Campbell (1918) a row of four or five cells is produced while in *Conocephalum* Boletter (quoted by Haupt 1926) reports 4–8 superimposed cells before vertical walls are laid. On the other hand, Durand (1908) in *Marchantia* and Abram (1899) in *Cryptomitrium* found three superimposed cells as in *Stephensoniella* before the appearance of the vertical walls.

In one case only an antheridium appeared to develop like mosses with a two-sided apical cell with segments cut off alternately right and left (Text-Fig. 10 d). This was the only case observed in spite of the fact that a number of sections were cut. Campbell (1918) describes occasional similar antheridium development in *Riccia glauca*.

The cells of the spermatogenous tissue divide vigorously forming a large number of small cubical cells with dense contents (Text-Fig. 10 f). Sometimes some of the basal cells of this tissue remain sterile so that the wall which is all round only single-layered is two or three layers of cells thick at the base.



TEXT FIG 10

Different stages in antheridium development and sperm formation

- a—Antheridium initial divided by oblique wall into a basal stalk cell and an upper cell. $\times 500$
- b—Formation of vertical walls after the division of the upper cell by a single transverse wall $\times 500$
- c.—Normal condition where the vertical walls are laid after the upper cell has divided into 3 superposed cells. $\times 500$
- d.—Abnormal case of development of antheridium with two sided apical cell as in mosses $\times 500$
- e and f—Later stages in antheridium development The stalk cell yet undivided. $\times 500$
- g—Different stages in the formation of sperm in spermatid. $\times 1067$

The spermatogenous tissue is broadly divided into compartments which are lineally related to the early formed spermatogenous cells. In each of these compartments all the cells within it are at the same stage of mitotic activity at any one time while those in any other compartment may be at another. Thus in one compartment all the cells may be at prophase, in another all at metaphase, in a third all at telophase, in a fourth all at the resting stage and so on. It is curious that the same factor or force governs the mitotic activity of all cells in any one compartment and has nothing to do with those in the other.

It was not possible to study spermatogenesis in detail. The sperm-mother cells, after the final division in the spermatogenous tissue, round off. Each divides as usual in Liverworts into two semicircular spermatids each containing a single nucleus in the centre. The nucleus gradually shifts on one side and comes into contact with the wall where it begins to elongate and curve along the wall (Text-Fig 10.g). Ultimately a sperm with two cilia at the apex, escapes from the spermatid. The origin of the cilia could not be observed.

The stalk cell of the antheridium ultimately divides by a few transverse and vertical walls giving rise to a short stalk. The wall of a ripe antheridium is very often compressed. Leitgeb (quoted by Campbell 1918) found in *Targionia hypophylla* walls so much compressed as to appear like a simple membrane. The antheridium at maturity may be pear-shaped, ovoid or flattened.

Female Receptacle—The female receptacle arises terminally as a small knob-like outgrowth in a pit situated between the two lobes of the thallus (Pt XXVIII, Fig 1). There is no anterior wall of the pit but posteriorly and laterally the walls bend inwards and protect the young receptacle. Further protection is afforded by the filamentous appendages of the scales of the terminal region of the thallus which closely cover it. A number of paraphyses or filaments three to six cells long arise from the base of the receptacle, grow upwards, and give additional protection.

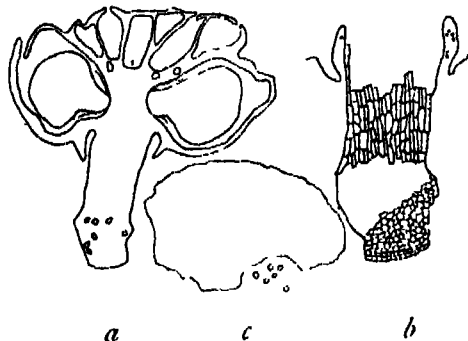
Even when the receptacle is very small just like a hemispherical knob, usually two processes or shoots are given off from the sub-apical region, one on either side of the female receptacle in front of the midrib on the under-side. In many cases they do not arise from the same point but are fairly apart from one another as regards their point of insertion on the midrib. Both of these lobes may be fully developed and equal in size (Text-Fig 1 a) or poorly developed but equal in size (Text-Fig 1 b) or unequal in size (Text-Fig 1 c) or one of these may give rise to a lobe, the other forming a tuber (Text-Figs 1 d, e, f) or both of these may give rise directly to tubers (Text-Fig 1 g). The formation of the lobes or their remaining undeveloped and directly forming tubers depends upon the seasonal conditions. If favourable, the lobes are formed, if unfavourable the tubers. Frequently only one process from one side of the sub-apical end is formed, the other remaining abortive. This again may be in the form of a lobe (Text-Figs 1 h, i, j) or in the form of a tuber (Text-Fig 1 k) but it is distinctly deflected on one side and is inserted only upon half the thickness of the midrib. In any case the apex of the plant after forming the female receptacle does not continue its growth.

forward as stated by Kashyap (1914) but one or two lobes are formed in the sub-apical region on the two sides. When only one of these lobes develops, it apparently gives the appearance as if it is a continuation of the apical region particularly when seen from the dorsal surface (Text-Fig 1 j). Thus the female receptacle in *Stephensoniella* does not become truly dorsal in position as described by Kashyap (1929) in the sense it does in *Plagiochasma articulatum* and *Preissia quadrata*. In the latter two species the apical region actually forms a lobe directly in front after forming the female receptacle.

An undivided female plant bears only a single female receptacle. Plants divided once or twice form as many female receptacles, one at the apex of each lobe.

The young receptacle bearing archegonia is sessile but at maturity when the sporogonia are being formed it becomes shortly stalked.

The structure of the receptacle is like that of the vegetative thallus. There is an upper row of large empty chambers arranged in a single layer and each opening by a single simple pore of the same type as in the thallus (Text-Fig 11 a). Below this is the ventral tissue composed of compact parenchyma with mucilage cells interspersed.



TEXT-FIG 11

- a—A V.S. of the female receptacle showing two involucres, a single layer of empty air-chambers and an annular groove at the junction of the stalk with the receptacle $\times 10$
- b—V.S. of the stalk of female receptacle. Tuberculate rhizoids which arise in the annular groove are cut transversely $\times 17$
- c—T.S. of the stalk of female receptacle with a single anterior groove. The rhizoids in this are cut transversely $\times 38$

There is an annular groove at the junction of the receptacle with the stalk and from this spring a few tuberculate rhizoids (Text-Fig 11 b where the rhizoids have been cut transversely and appear as small circles).

The stalk at maturity is 1.5 mm long. It is more or less flattened at the base and cylindrical above. There is only a single groove or furrow in the anterior position as shown in a transverse section (Text-Fig 11 c) from where are given out only tuberculate rhizoids. The tissue of the stalk is parenchymatous. The air-chambers are absent. The cells at the base of the stalk are small and isodiametric while those in the upper region are large and elongated in the direction of its long axis (Text-Fig 11 b).

The receptacle has two lateral groups of archegonia—one on each side and each group is enclosed in an involucre. There are 1–4 archegonia in each group. Kashyap (1914) describes upto six archegonia in each involucre.

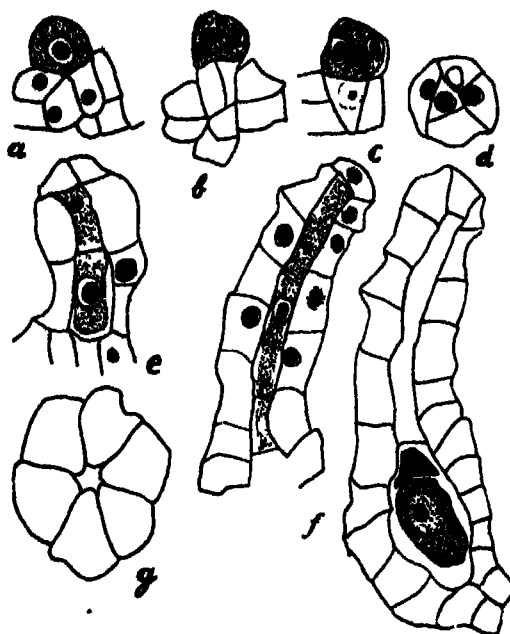
The origin of air-chambers in the female receptacle is exactly on the same lines as in the thallus.

Archegonium development—The archegonia develop on the morphologically upper surface of the receptacle in an acropetal succession behind the two growing points but get shifted on the undersurface by the greater growth of tissue on the dorsal surface.

A superficial cell projects as a papilla from the upper surface and contains dense cytoplasm. A transverse wall in level with the surface of the thallus or slightly above it cuts off an inner stalk cell and an outer archegonium initial (Text-Fig 12 a).

According to Strasburger in *Marchantia* and Janczewski in *Preissia commutata* (quoted by Campbell, 1918) this outer cell again divides by a transverse wall of which the inner forms the foot of the archegonium while the outer gives rise to the archegonium itself. Campbell (1918) did not find it in *Targionia* and *Fimbriaria*, Ahmed (1938) did not observe it in *Aitchisoniella* nor did Abram (1899) report it in *Cryptomitrium* nor Durand (1908) in *Marchantia polymorpha*. In *Stephensiella* also this is not the case.

Further development of the initial is as in other Marchantiales. It divides by a rather curved vertical wall into two unequal cells (Text-Fig. 12 b). In the bigger cell another vertical wall appears followed by another similar one, each of these cutting the other two. Thus an axial cell becomes enclosed by three peripheral cells (Text-Figs 12 c, d). The stalk cell divides by a vertical wall even at the stage when the first vertical wall appears in the archegonium initial (Text-Fig. 10 b). Each of the three peripheral cells divides into two by a vertical radial wall. Thus 6 cells are formed which are called the primary wall cells. Occasionally 7 may be formed by the further division of any one of these by another vertical radial wall. In the meantime the axial cell cuts off an upper smaller cap cell or cover cell and a



TEXT-FIG 12

Archegonium development All $\times 500$

- a.—Archegonium initial divided into a lower stalk cell and an upper cell
- b.—First vertical oblique wall laid in the upper cell The stalk cell also divided by a vertical wall
- c.—Three vertical oblique walls formed in the upper cell, each cutting the other two, forming an axial cell and 3 peripheral cells Only two of the latter are seen in a longitudinal section
- d.—Same as c in T.S.
- e.—Cap cell divided, besides a primary neck canal cell and a primary ventral cell differentiated
- f.—An archegonium in two sections showing 5 neck canal cells, a ventral canal cell and an egg
- g.—T.S. of the archegonium neck showing 6 cells enclosing the canal

lower larger cell The latter divides by another transverse wall into an upper primary neck canal and a lower primary ventral cell (Text-Fig 12 e) At this time the cover cell is divided into four cells by two vertical walls at right angles to one another, which close the archegonium In a vertical section at this stage only two cells are visible (Text-Fig 12 e) Any one or more of these may divide further by vertical radial walls and increase the number of cap or cover cells. In the meantime each of the primary wall cell divides transversely (Text-Fig. 12 e) The upper cells are the primary neck cells each

of which by successive transverse divisions form a row of upto 16 cells. Thus the neck is formed of 6-7 rows of neck cells with upto 16 cells in each row. The lower cells divide by transverse and vertical radial walls forming wall of the venter which is one layer of cells thick. The primary neck canal cell also divides by a number of transverse walls forming 5-8 neck canal cells (Text-Fig 12 f). The primary ventral cell divides once to form a ventral canal cell and an egg (Text-Fig 12 f).

The neck of a fully mature archegonium is more or less twisted. The venter is at first one layer of cells thick but after fertilization becomes 2-3 layers thick by periclinal division of its cells.

As usual, on ripening of the archegonium the cap cells diverge and the neck canal cells and ventral canal cell disintegrate to form a thick mucilaginous fluid.

Sporophyte

Embryo Development—The egg after fertilization enlarges in size and secretes a wall around itself. The first wall is transverse forming an upper epibasal and a lower hypobasal cell (Text-Fig 13 a). A vertical wall is next formed in each of the two cells resulting in a quadrant. This is followed by two other vertical walls at right angles to the first vertical wall in both forming an octant (Text-Fig 13 b).

Sometimes the first wall is oblique. The next vertical walls in each of the two halves are also obliquely laid but they are at right angles to one another and to the first wall.

Occasionally the first wall is not transverse but vertical and a quadrant is formed by subsequent transverse walls in each of the two cells (Text-Fig 13 c).

In one abnormal case two successive transverse walls were formed giving rise to three superimposed cells followed by vertical walls in the upper cells (Text-Fig 13 d). This comes very near to the 'filamentous type' of embryo.

The genera of the Marchantiales so far worked out are arranged as follows on the basis of embryo development —

Octant Type

<i>Marchantia polymorpha</i>	..	Durand, 1908
<i>Marchantia domingensis</i>	..	Anderson, 1929
<i>Preissia quadrata</i> . . .	.	Haupt, 1926
<i>Fegatella comica</i>	..	Cavers, 1904
<i>Cryptomitrium tenerum</i>	..	Abram, 1899

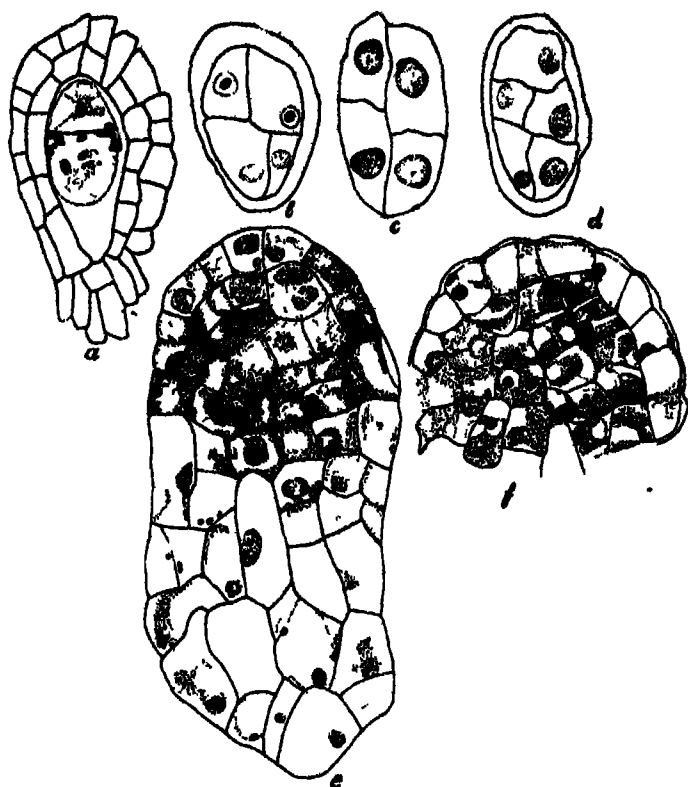
<i>Fimbriaria californica</i>	.	.	Campbell, 1918
<i>Grimaldia</i> sp	.	.	Kienitz-Gerloff, 1874
<i>Targionia hypophylla</i>			Campbell, 1918
<i>Cyathodium foetidissimum</i>	.	.	Lang, 1905
<i>Ricciocarpus natans</i>	.	..	Garber, 1904
<i>Riccia lutescens</i>	.		Lewis, 1906
<i>Riccia frosteri</i>	.		Black, 1913

Filamentous Type

<i>Fegatella conica</i>		Boletter, 1927
<i>Aitchisoniella himalayensis</i>		Ahmed, 1938
<i>Reboulia hemispherica</i>		{ Woodburn, 1920 Haupt, 1921 Dupler, 1922
<i>Plagiochasma</i>	.	{ Meyer, 1913 Starr, 1916
<i>Targionia hypophylla</i>		O'Keefe, 1915
<i>Cyathodium cavernarum</i>		Lang, 1905
<i>Ricciocarpus natans</i>		Garber, 1904

It is evident from above that even in the same species *Targionia hypophylla* and *Ricciocarpus natans* both types of embryo development occur. Also in different species of the same genus *Cyathodium* worked by Lang the two types of embryo development are observed.

There is no regularity in the subsequent cell divisions and a multicellular embryo with more or less similar cells results. There is no formation of a two-sided apical cell such as was figured by Hofmeister for *Targionia* and described by Woodburn (1920) for *Reboulia*. The cells then divide with greater activity in the upper region than in the lower ultimately differentiating an upper region with small cells and dense contents from the lower with large cells and lax contents. At this stage the lower region is larger than the upper but after a short time both become of almost equal size (Text-Fig 13 e). At the same time a constriction appears in the middle of the rudimentary embryo where the cells do not divide actively. This constriction marks the region seta separating the upper capsular region from the lower bulbous region or the foot. In living teased out embryos the small cells of the upper region are full of chloroplasts and contrast markedly with the foot cells with but few chloroplasts. This stage appears about the last week of July at Mussoorie.



TEXT-FIG. 13

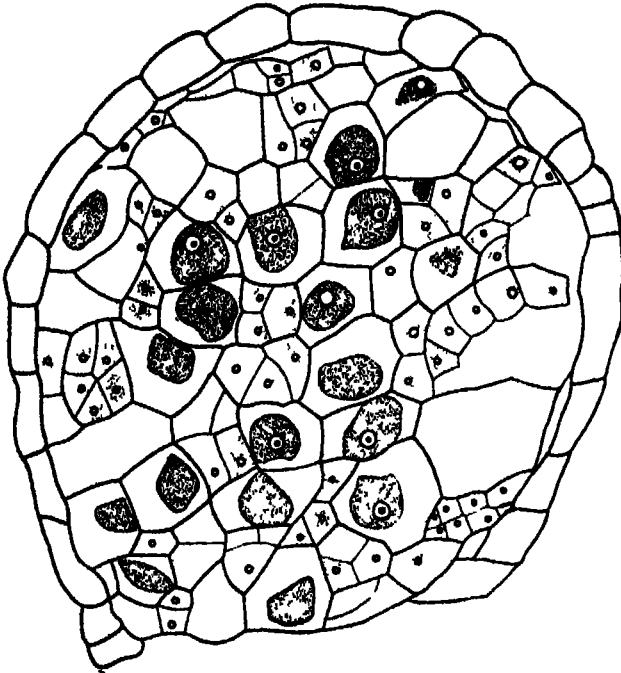
Development of the embryo. All $\times 875$

- a.—First transverse wall in the egg forming an upper epibasal and a lower hypo-basal cell
- b.—Quadrant stage
- c.—Fertilized egg in which the first wall was vertical followed by transverse walls giving rise to a quadrant
- d.—Fertilized egg divided by 8 successive transverse walls. Vertical walls are formed in the two upper cells
- e.—Embryo where the differentiation into an upper capsular region of short cells and a lower foot region of large cells has occurred. The constriction marks the position of the seta
- f.—Differentiation of an outer one layered amphithecium from inner endothecium

The cells in the upper region divide vigorously. By the formation of periclinal walls in the peripheral cells an outer single layer of cells—the amphithecium is cut off from the central tissue or the endothecium rather early (Text-Fig 13 e, f). The cells of the amphithecium gradually enlarge and lose their contents and divide by radial walls to keep pace with the

enlarging endothecium within The cells of the latter divide actively and are densely packed with chloroplasts and food grains

Finally spore-mother cells are differentiated and the contents in these begin to round off Not all the cells become transformed into spore mother cells Some of these interspersed among the mother cells divide further, each giving rise to form 3-5 smaller cells (Text-Fig 14) The smaller cells are the



TEXT-FIG 14

Differentiation of the archesporium into spore-mother cells and elator mother cells. Large cells belonging to the same generation as spore-mother cells have divided into 3-5 small cells each of which is the elator mother cell. $\times 360$

elator mother cells and form the elators It is obvious therefore, that the spore mother cells and the elator mother cells do not belong to the same cell generation in *Stephensoniella*

In *Marchantia polymorpha* studied by Durand (1908) and O'Hanlon (1926) and *Marchantia domingensis* by Anderson (1929), elator mother cells are differentiated earlier than the spore mother cells in the archesporium and are homologous with a row of spore-mother cells In *M. polymorpha* according to O'Hanlon they are differentiated five generations before the spore-mother cells and in *M. domingensis* 3-4 generations before the spore-mother

cells In *Reboulia hemispherica*, Haupt (1921) found that the spore-mother cells and the elator mother cells belong to the same cell generation But in *Stephensoniella*, the elator mother cells belong to 3-5 cell generations later than the spore-mother cells These facts are clearly correlated with the size of the mature elators in these genera In *Marchantia* they are the largest ($540\text{ }\mu$ - $630\text{ }\mu$), intermediate in *Reboulia hemispherica* ($300\text{ }\mu$ - $400\text{ }\mu$) and smallest in *Stephensoniella brevipedunculata* ($80\text{ }\mu$ - $140\text{ }\mu$) * Thus the earlier the differentiation of the elator mother cells in the sporogenous tissue relatively to the spore-mother cells the larger is the size of the elators and later their differentiation the smaller is their size The shifting off of the elator mother cells to later generations with respect to spore-mother cells in *Stephensoniella* is distinctly a reduced feature compared to that in either *Marchantia* or *Reboulia* and the series runs from *Marchantia* through *Reboulia* to *Stephensoniella*

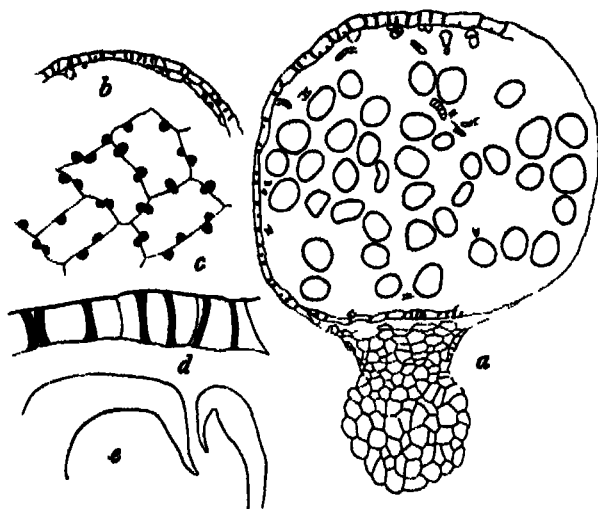
In the spore-mother cells, the protoplast withdraws itself from the original wall and assumes different 'amæboid' forms as in *Reboulia* described by Haupt (1921) At the same time a wall is formed outside the protoplast which stains blue with Delafield's Hæmatoxylin (Text-Fig 14) The original wall of the spore-mother cells gradually disintegrates

There is no lobing of the spore-mother cell prior to meiotic and mitotic divisions The mother cells are densely stored with food material The spores are formed by the usual tetrahedral division (Text-Fig 16 a)

Mature sporogonium—The sporogonium on maturity becomes more or less horizontal and is more or less included within the involucre One or more sporogonia may start their development within an involucre but usually only one matures In rare cases two sporogonia mature in an involucre (Text-Fig 1 g) Frequently fertilization does not occur and consequently the involucre remains empty.

Each lip of the two lipped involucre is 3-5 layer of cells thick becoming single-layered at the margin There are no air-chambers present in the involucre tissue In early stages the front margins of the two lips are bent inwards parallel to one another towards the cavity where the sporogonia are lodged leaving a slit-like opening (Text-Fig 15 e) This type of aestivation of involucre lips has been observed by Kashyap (1914) in *Plagiochasma appendiculatum*, *P. articulatum*, *P. Aytonia* and *Reboulia hemispherica*

* The average size of the spores in *Marchantia polymorpha* is $12\text{ }\mu$, in *Reboulia hemispherica* is $70\text{ }\mu$ and in *Stephensoniella brevipedunculata* is $90\text{ }\mu$ The size of the elators with respect to their spores is therefore approximately 48:1 in the first, 5:1 in the second and 1:1 in the third.



TEXT-FIG 15

- a*—Mature sporophyte with bulbous foot, short seta and capsule $\times 53$
b—Upper portion of the capsule wall For some part it is 2 layers of cells thick
 Short elator-like cells attached as in '*a*' $\times 53$
c and *d*—Capsule wall in tangential and radial sections respectively, showing thickened bands only along the radial walls $\times 240$
e.—Aestivation of the involucrel lips They are turned inwards leaving a slit-like opening $\times 33$

A mature sporogonium consists of a bulbous foot, a very small seta represented by a constriction and a large capsule (Text-Fig 15 *a*)

The cells of the foot are haustorial in function and large (Text-Fig 15 *a*) They destroy the tissue of the receptacle below leaving a mucilaginous deposit and absorb the food material No haustorial tubes like those described by Land (1905) for *Cyathodium* are formed from these cells in *Stephensoniella*

The cells of the seta are short and isodiametric

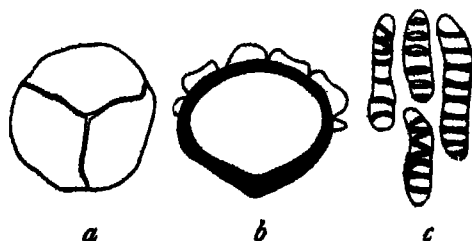
The wall of the capsule is single layer of cells thick except at the apex where frequently it may be two layers thick (Text-Fig 15 *b*) In ripe sporogonia the cells of the wall possess thick brown bands on the radial walls only and none on the tangential walls A part of the wall on a magnified scale in both the tangential and radial sections is shown in Text-Figs 15 *c*, *d* respectively These thickenings develop after the tetrad stage

The elators are rather short 80μ – 140μ in length and possess mostly annular bands or a single loose spiral one or sometimes a part of the elator may possess annular band and the rest a short spiral one (Text-Fig. 16 *c*)

As remarked by Kashyap (1914) the capsule wall has at its apex towards the interior a few short elator-like cells which possess annular or spiral

thickenings (Text-Fig 15 *a, b*) Similar cells have been observed by Kashyap (1914) and O'Keefe (1915) from both the apex and the base of the capsule in *Targionia* and by Kashyap (1914) and Ahmed (1938) in *Aitchisonella* from the apex only. Cavers (1911) figures a number of fixed elator-like cells in *Preissia quadrata* both from the apex and the base of the capsule

Spores—Spore is tetrahedral in shape. In a transverse section the wall shows a thin intine, a moderately thick darkish brown exine and a thick yellowish perisporium. On the outer circular wall only (not on the flat walls of the tetrahedral spore) fringe-like processes or lamellæ are present which seem to arise from the exine but grow further out beyond the perisporium (Text-Fig 16 *b*)



TEXT-FIG. 16

a.—A tetrad. $\times 240$

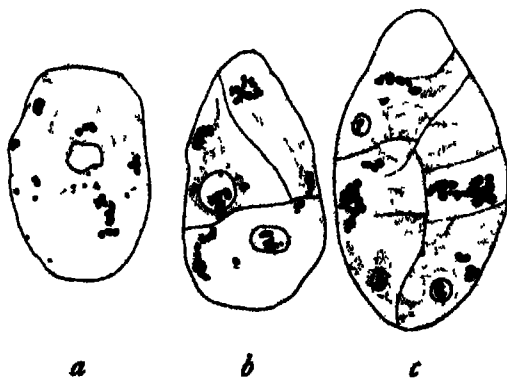
b.—Mature spore with thin intine, a moderately thick exine and a perisporium.

Fringe-like processes are developed only on the peripheral circular wall. $\times 240$

c.—Elators $\times 240$

Spherules

Some interesting bodies of the nature of small spheres which stain jet black with iron alum hæmatoxylin have been observed in the fertilized egg and the succeeding stages of its division. Apparently they look like mitochondria but in the absence of proper tests they are provisionally named as 'spherules' (Text-Fig 17)



TEXT-FIG. 17

a, b and *c*.—Fertilized egg and its subsequent stages of division with dark coloured spherules suggestive of mitochondria. $\times 543$

These may be singly scattered or more frequently grouped in an irregular manner or arranged in the form of chains. Quite commonly they are distorted and that is probably due to the action of the fixative. In many cases the adjacent spherules in a chain or in a group are fused forming irregular densely staining masses.

On the first division of the fertilized ovum there appears to be neither any division of the individual spherules nor any orientation on their part in preparation to the oospore division. They simply stay where they are and consequently in each of the two cells formed the number is only approximately halved. During the further divisions of the two cells the same process is repeated until in the octant stage a few of these bodies are visible in each cell. They persist in the embryo a little beyond the post-octant stage after which they seem to disappear.

Chromosome Number

The haploid chromosome number in the species is 9. This is observed both in a dividing vegetative cell nucleus in the thallus (Text-Fig 18 a), and



TEXT-FIG. 18

a.--Nine chromosomes at metaphase plate in a vegetative cell $\times 3200$

b --Nine chromosomes at metaphase plate in a spermatogenous cell $\times 3200$

in the dividing nuclei of the spermatogenous cells in antheridia (Text-Fig 18 b), in both of which the chromosomes are arranged in the form of a plate at the metaphase.

Discussion

Summing up the chief features of the genus *Stephensoniella*, we find that the structure of the thallus is not very complex. There is a single row of large, empty, obliquely directed forward air-chambers. The pores are of the simplest type. The antheridia are aggregated together to form a male receptacle of the simplest type on the median dorsal surface of the thallus. The female receptacle is terminal in position and shortly stalked at maturity. There are only two involucres with 1-4 archegonia in each. The pores of

the chambers of the female receptacle are also simple. The stalk of the receptacle has only a single shallow groove possessing a few tuberculate rhizoids. The sporogonium is very simple with a bulbous foot and short seta. The wall of the capsule is single-layered, sometimes two-layered at the apex and the cells possess thickened bands on their radial walls. The elators are short and possess either annular thickenings or a single spiral band. Sometimes a few elator-like cells project into the capsule cavity from the apex.

Kashyap (1914) places it near the genus *Exormotheca*. There is no question about the close affinity between the two genera.

There is a general resemblance in the external form. Both the plants are of tuber forming habit. In both the pores on the thallus and female receptacle are simple and there is a single layer of air-chambers. The position and the structure of the male receptacle is the same. In both cases the young female receptacle is situated in a pit in the angle between two vegetative shoots. In both, this pit has no anterior wall but posteriorly and laterally the walls bend inwards and protect the young receptacle. The stalk of the female receptacle in both has a single furrow. Both resemble one another in the number and position of the involucre in the female receptacle and also in the number of archegonia in each involucre. The structure and position of sporogonium in both is quite similar. There are short elator-like cells attached at the apex of the capsule wall and projecting into the interior in both.

Stephensoniella, however, differs from *Exormotheca* in being dioecious, in its spongy texture, in the absence of filaments in the air-chambers, in the presence of rather shallow groove in the stalk of the *Carpoccephalum* (in *Exormotheca* it is deep) with only a few tuberculate rhizoids; in the shorter stalk of the female receptacle, in the formation of two sub-apical lobes (frequently only one) after the formation of the female receptacle and in the simpler thickenings on the wall cells of the capsule and the elators (in *Exormotheca* in the upper half of the capsule wall four or five bands start from the centre of the inner tangential wall and extend to the radial walls while in *Stephensoniella* there are no such bands on the inner tangential walls of the wall-cells of the capsule). Again the elators in *Exormotheca* are comparatively large and possess three or four lax spiral bands in contrast to a single spiral band, when such is present, on the short elators in *Stephensoniella*.

It is therefore clear that while *Stephensoniella* finds its natural place in the phyllogenetic series with *Exormotheca*, thus confirming the position already assigned to it by Kashyap (1914), it has a comparatively simpler organisation.

The question next arises, is it from *Stephensoniella* that a form like *Exormotheca* has arisen by further elaboration or the former has been derived from ancestors like the latter through a process of reduction

A comparison of the groove in the stalk of the female receptacle in the two genera affords a feature of some value. The groove in *Stephensoniella* is very shallow and contains but a few rhizoids in contrast to the deep groove and large number of rhizoids in the stalk of the female receptacle in *Exormotheca*. It is now generally recognised beyond any question that the stalk of the female receptacle in Liverworts is a modified vegetative branch of the thallus. The rhizoids borne on the groove are the remnants of those on the ventral surface of the thallus and have now become useless with the vertical growth of the stalk of the receptacle. If there has been a process of elaboration in evolution and the complex form derived from the simpler one, there would probably be some biological advantage justifying the deepening of the groove and consequently formation of a larger number of rhizoids from shallow grooved ancestors like *Stephensoniella*. Is there any such advantage visible? Obviously there is none. On the other hand the derivation of shallow groove from deep groove can be easily explained. The rhizoids being of no use on account of the vertical position of the receptacle stalk, the groove which bears them is of no consequent advantage and becomes shallow. This feature, though by itself fragmentary, but taken in conjunction with a large mass of evidence in support of a wholesale reduction in the evolution of Marchantiales demonstrated by Kashyap (1919) certainly has got its own value and suggests *Stephensoniella* to have been derived from ancestors like that of *Exormotheca* through a process of reduction.

Position of the female receptacle—Kashyap considered the female receptacle to be terminal in position to begin with, later becoming dorsal by continued growth of the apex. The present observations show that there is no distinct terminal lobe formed after the formation of the female receptacle as occurs in *Plagiochasma articulatum* or *Preissia*. On the other hand there are two sub-apical shoots formed, one on either side of the female receptacle and in many cases they are fairly apart from one another as regards their point of insertion on the midrib. One of these may commonly be completely suppressed. Whether or not we regard these two shoots as the two lobes of an apical shoot which has dichotomised at the point of its origin is merely a matter of opinion. If we assume that these lobes are those of the terminal shoot, formed after the formation of the female receptacle, which has bifurcated at its very start, the latter is truly dorsal in position; on the other hand they certainly can be regarded as merely sub-apical lobes.

corresponding to the ventral shoots in other forms, particularly when in many cases these lobes have not a common point of origin and are fairly apart from one another in the matter of their insertion on the midrib of the main thallus. Again we have observed that one of these sub-apical shoots may quite commonly be completely suppressed while the other which grows is deflected to one side. A slight shifting of this remaining shoot in the middling position would make the female receptacle actually dorsal in position. In any case while we cannot call the female receptacle in *Stephensoniella* as strictly dorsal in position as it is in *Plagiochasma articulatum* and *Preissia* there certainly seems to be a tendency towards an approach in that direction.

The position of *Stephensoniella*, therefore, as a probable link from *Exormotheca* like ancestors to *Corsinia* and *Boschia* on Kashyap's line of evolution by reduction, while feasible on a comparison in other features, goes a step backwards in view of the present observations which do not support the assumption of a truly dorsal position of the female receptacle in the life-history of the species.

Presence of chlorophyll in the sporogonium—It has usually been recognised that the sporogonia of Anthocerotales only are capable of manufacturing certain amount of carbohydrates because of the presence of chlorophyll in their cells and are therefore semiparasitic while the sporophytes of the Marchantiales, Jungermanniales and Sphaerocarpaceae are completely parasitic. In a recent contribution Bold (1938) showed the presence of chloroplasts in the developing sporogonia of 5 species of the Marchantiales and some 3 species belonging to the Jungermanniales and pointed out a certain degree of nutritional independence in the sporophytic generation of these two groups. It has been shown above that the living teased out sporogonia in early stages of development in *Stephensoniella* showed that the cells particularly of the upper region which later form the capsule are packed up with chlorophyll granules. This certainly suggests at least a partial manufacture of the carbohydrate food material and adds another instance to show that the sporophytic generation of the Marchantiales is not completely parasitic but on a par with the Anthocerotales in manufacturing to a certain extent organic carbohydrates although in the latter there is a greater amount of vegetative tissue containing chlorophyll set apart for this purpose and there are definitely organised stomata for gaseous exchange.

Summary

The plant is strictly dioecious. The thallus is dorsiventral, spongy in texture and grows by an apical cell.

There is an upper single layer of large, empty air-chambers which are obliquely directed forward and which open by simple pores and a lower compact parenchymatous tissue in which mucilage cells are interspersed. No mycorrhiza is observed in this tissue.

Origin of air-chambers is schizogenous as described by Barnes and Land.

Vegetative reproduction takes place by means of adventitious ventral shoots or tubers—the latter formed at the end of the vegetative period for perennation. The structure of the tuber is merely that of a condensed thallus. The air chambers are present and not absent as described by Kashyap. The ventral tissue is packed with starch.

An interesting point is that the tubers from male plants produce antheridia which become arrested in different stages of their development on the arrival of unfavourable conditions and complete their development in the next vegetative season. Thus they complete their development in two seasons.

Antheridia are formed in definite receptacles on the dorsal surface. Their development and structure is similar to those in other Marchantiales.

The female receptacle is terminal, never becoming strictly dorsal in position as it does in *Plagiochasma articulatum* and *Preissia*. There are two subapical shoots formed after the formation of the female receptacle. Quite commonly one of these shoots may be suppressed.

There are two lateral involucre to each receptacle, with 1–4 archegonia in each involucre. The receptacle has a single layer of empty air-chambers opening by simple pores as in the thallus. There is a single anterior groove in the receptacle stalk bearing only tuberculate rhizoids.

The archegonium has a twisted neck formed of 6 or 7 rows of neck cells with upto 16 cells in each row. There are 5–8 neck canal cells. The development of archegonium is as usual in the Marchantiales.

Embryo development is of the octant type. The sporogonium at maturity consists of a bulbous foot, a constriction-like seta and a capsule.

The cells of the living sporogonium in early stages of its development are packed with chloroplasts, particularly in the capsule region. It is, thus, capable of manufacturing the carbohydrate food material to a certain extent and therefore semiparasitic, resembling in this respect the sporogonia of Anthocerotales.

The elator mother cells belong to 3-5 generations later than the spore-mother cell generation. In this respect *Stephensoniella* shows reduction as compared to either *Marchantia* (where they are differentiated earlier than the spore-mother cells) or *Reboulia* (where both belong to the same cell generation). Also in the Marchantiales in different genera the earlier or later differentiation of the elator mother cells relatively to the spore-mother cells effect the size of the mature elators.

The capsule wall at maturity is one-layered and sometimes two-layered at the apex only. A few elator-like cells are attached at the top of the capsule wall and project inwards. The wall cells have thick brown bands on their radial walls only.

The elators are short with annular bands or a single spiral band. The spores are tetrahedral and lamellate on the outer margin only.

Round bodies or spherules suggestive of mitochondria have been observed in the fertilized egg and its subsequent divisions until a little after the octant stage, after which they are no longer seen.

The haploid chromosome number is 9.

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FIG 1



FIG 2



FIG 3

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EXPLANATION OF PLATE XXVIII

- FIG. 1.—L S of thallus showing the young female receptacle at the apex
- FIG. 2.—L.S of the tuber. The cells of the ventral tissue are full of starch Antheridia developing in an acropetal succession from the apex backwards Air-chambers present on the upper surface
- FIG. 3.—Ventral region of the thallus in L.S showing dark mucilage cells interspersed

A PECULIAR CASE OF CAUDAL ABNORMALITY IN *HEMIDACTYLUS FLAVIVIRIDIS* RÜPPEL

BY MAHESHWAR SINGH SOOD, M Sc

(From the Department of Zoology, St. John's College, Agra)

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(Communicated by Mr Beni Charan Mahendra, F z s)

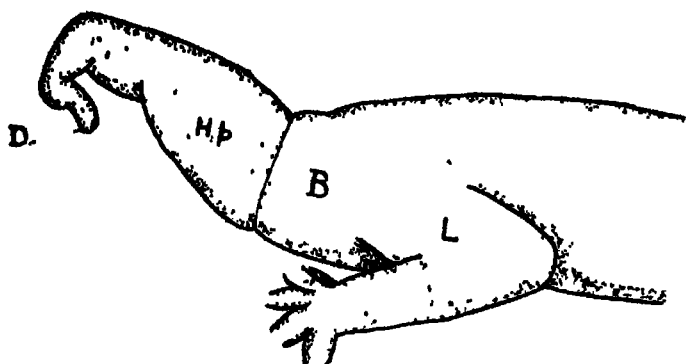
Introduction

THE occurrence of bifid or trifid tails in lizards has been noted by many authors. Thus, for example, Giebel (1864) recorded a case of *Lacerta agilis* with a supernumerary tail growing on the ventral side of the normal tail. Quelch (1890) described a specimen of *Tupinambis nigropunctatus* Boul with a bruised tail which developed three large supernumerary tails and three small stumps between them. Tornier (1897) dealt with the experimental production of trifid tails in lizards. Brindley (1894, 1898) reported on several cases of multiple tails belonging to eight species (*Hemidactylus gleadowi*, *Calotes cristatellus* Fitz., *Anolis grahami* Gray, *Lygosoma telfairi*, *Trogonophis weigmanni*, *Lacerta agilis*, *L. muralis*, and *Mabuya carinata*). Stuart (1908) observed an individual of *Hemidactylus gleadowi* bearing a straight tail with two supplementary pieces attached on the left side. Loveridge (1920, 1923) noted two bifid-tailed specimens of *Hemidactylus mabouia*, but did not give further details about them. Woodland (1920) and Slotopolsky (1921) studied the processes of caudal autotomy and regeneration, as well as the causation of tail abnormalities in lizards. Hora (1926) figured a case of *Hemidactylus brooki* with a tri-radiate tail. Das (1932) gave a detailed account of two trifid-tailed individuals of *Hemidactylus flaviviridis* and experimented on the artificial regeneration of such tails in *Gecko verticillatus* Laur.

The specimen studied in the present paper, however, differs from all the previous records and does not belong to the bifid or trifid types. Its structure is unique in many ways and deserves a detailed description. It was obtained by Mr Beni Charan Mahendra during class dissections last year and was handed over to me for a detailed study.

General Account of the Caudal Region

The tail of the specimen under consideration (Text-Fig 1 and Plate XXIX) can be divided into three regions: (1) an unsegmented basal region, (2) a large regenerated hook-shaped structure, distinctly consisting



TEXT-FIG 1

Lateral view of the abnormal tail of *H. flaviviridis* Rüppel

B the basal region, *D*, the distal extremity of the hook-shaped region, *Hp*, the hook-shaped region, *L*, the right thigh

of a right and a left part, and (3) a small bud-like part visible ventrally on the left side

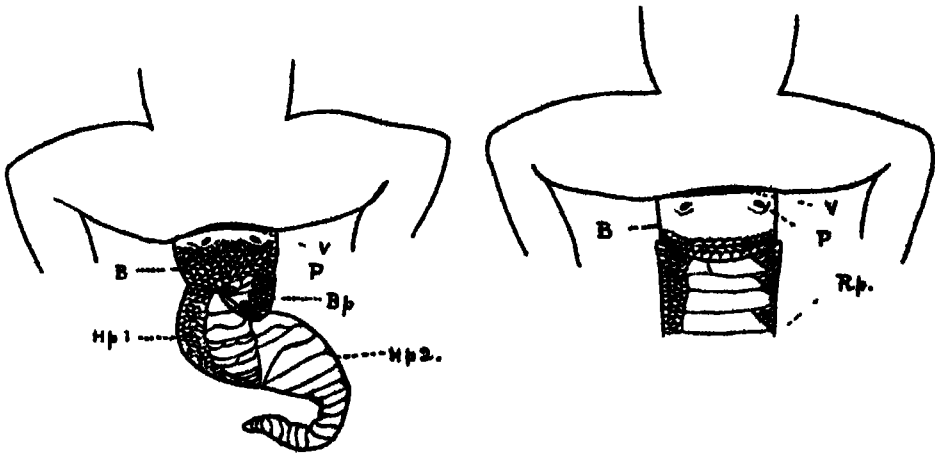
The basal region is quite normal both on the dorsal and the ventral sides

The abnormal regenerated tail is attached to the basal portion, has got a hook-like appearance with the concavity directed towards the right side, and is not in straight alignment with the normal basal part but is directed, on the whole, upwards and lateralwards. The free extremity of the hook points downwards towards the right and is distinctly curled distally. The base of the regenerated part is almost as wide (9 mm) as the normal basal segment and can be readily distinguished from the original caudal region by a furrow, which passes all round this region. The dorsal surface bears also an ill-defined median furrow distinguishing the right part from the left. The right part is the smaller of the two and forms half of only the base of the hook as it is not continued distally. The left part forms half of the basal region and the whole of the distal part of the hook, it tapers markedly towards the tip. The line of demarcation between the two regions is not so marked on the dorsal side as on the ventral.

The bud-like portion can be seen only in a ventral view, lies attached to the left half of the basal segment and is only 3.5 mm long and 6 mm broad. It is marked off both from the basal region and the hook-shaped portion by furrows.

The Scalation of the Abnormal Tail

A. The basal portion (Text Fig 2, A and B) shows almost the same scalation both dorsally and ventrally as is found in a normal tail at this region,



TEXT-FIG. 2

- (A) Ventral view of the abnormal tail of *H. flaviviridis* Rüppel, to show the scalation
 (B) Ventral view of a normally regenerated tail for the sake of comparison;
B, the basal region, *Bp.*, the bud-like portion, *Hp₁*, *Hp₂*, the right and the left halves of the hook-shaped region respectively; *P*, post-anal aperture; *V.*, vent

On the dorsal side there is a uniform covering of minute subequal granular scales, while on the ventral the scales are slightly large and polygonal and there are two comparatively enlarged mid-ventral scales just at the posterior border of the segment

B Dorsally, the *hook-shaped region* is covered with minute granular scales interspersed with larger ones. Ventrally, it is distinctly marked off into a right and a left half, both of which possess a series of transversely enlarged plates, roughly resembling in appearance and disposition the mid-ventral scales of a normally regenerated tail

C The small *bud-like part* visible on the ventral side has no enlarged mid-ventral scales of the usual type. The small scales bounding it are arranged in concentric rings diverging from a point situated almost in its centre on the ventral side, and bordered by a large concentric scale both anteriorly and mesially

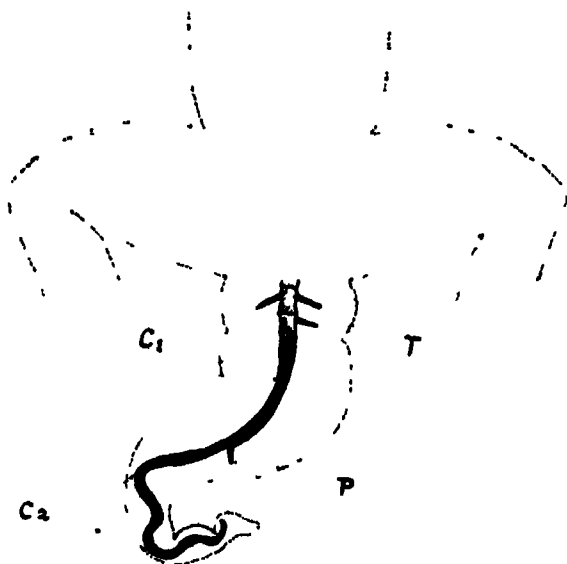
The Musculature

An attempt was made to study the arrangement of the muscle bands in the caudal region after the removal of the skin. However, the outlines of the individual bands were not distinct. The basal region was constituted as in an original tail of *Hemidactylus*, but the hook-shaped regenerated region had fairly numerous delicate muscles running longitudinally right up to the tip of the tail and showing no transverse divisions corresponding to autotomy septa

The Skeleton

The skeleton was studied by the alizarin method. The whole specimen was first treated with 1% potassium hydroxide solution for three days and then stained with alizarin stain (1 gm of alizarin dissolved in 1,000 c c of 1% potassium hydroxide solution). The superficial stain was removed by keeping the specimen again in 1% KOH solution, and clearing was done in glycerine. By keeping the specimen against strong light, the skeleton could be made out with fair facility.

The basal region (Text-Fig 3) had the normal type of skeleton with the usual vertebræ inside, but, curiously enough, the last vertebra lacked the transverse process on the left side.



TEXT-FIG 3

The skeleton of the abnormal caudal region of *H. flaviviridis* Rüppel (Dorsal view)

The regenerated part of the endo-skeleton is coloured black in order to distinguish it from the vertebræ

C₁, the proximal part of the cartilaginous tube, C₂, the distal, twisted portion of the cartilaginous tube, P, process of the cartilaginous tube on the right side, T., transverse processes

The cartilaginous tube of the regenerated part followed immediately after the last autotomised half-vertebra, but instead of following it backwards in a straight line, it curved to the left, giving off a short blunt process to support the right half of the hook-shaped region. After reaching very near to the left periphery of the regenerated region, it bent to the right forming a short loop and then passed on towards the tip of the tail, sinuously

bending on itself twice. An examination of the specimen in a lateral view showed that all the parts of the cartilaginous tube were not in the same horizontal plane, but each loop-shaped lateral twisting was also accompanied by a marked sinking downwards, so that the side loops also formed depressions facing upwards.

The small bud-like part of the tail on the ventral side had no skeletal structure inside.

I am unable to account for the peculiar sinuously twisted cartilaginous tube found in this unique specimen. Such a skeleton of the regenerated tail has not so far been recorded, and it is impossible at present to ascribe it to any definite cause.

Discussion

Although, I find it impossible to account completely for the unique abnormality found in this specimen, certain facts emerge as the result of this study.

(1) The basal region, as indicated by its scalation and skeleton, is evidently a part of the original tail.

(2) The ventral bud-like part is due to a slight injury on this region, affecting only the body-wall and the musculature. The absence of a skeletal support inside indicates that the injury was very superficial and did not reach up to the vertebral column, the small size of the regenerated growth seems to show that the injury was probably inflicted much later than that leading to the regeneration of the hook-shaped region.

(3) The hook-shaped region, as shown by scalation, seems to consist of two parts, viz., the right and the left, of different ages. Of these, the right perhaps represents an earlier growth. Its scalation corresponding to the right half of a normally regenerated tail seems to support such a view. The slight displacement of the cartilaginous tube from a straight alignment with the original vertebral column may not be an initial condition, but may have been produced by the unequal growth of the tissues around it after the longitudinal injury described below was inflicted.

(4) The bifurcation of the cartilaginous tube to form a short process on the right side and a long sinuously disposed limb on the left is probably due to a longitudinal injury affecting a normally regenerating tail in such a way that the left half of this tail completely broke away, while the right remained attached to the basal region. This is why the ventral shields on the left half appear to be halves of what we get in a normally regenerated tail and are bounded only on one side by minute scales.

(5) This longitudinal injury was probably not exactly along the mesial line, but was, on the whole, to one side of the cartilaginous tube and affected only the distal part of the cartilaginous tube, dividing it in an oblique manner. The sinuous development of the left distal part of the cartilaginous tube may be due to this oblique split. Woodland (1920) has already shown that the axis of the growing tail tends to be at right angles to the plane of the original cut and has figured an analogous case (cf Woodland, Text-Fig 5, C, C' p. 90) in which the regenerating tail underwent a hook-shaped curve pointing forwards.

Summary

The case of caudal abnormality described here is the first of its type and differs from all the previous records of such abnormalities in lizards. A complete account of its external appearance, scalation and skeleton is given, as well as some observations made on the musculature of the regenerated region. The author tries to interpret, as far as possible, how the growing tail must have been injured to produce such a peculiar abnormality. The cartilaginous tube is of a sinuous type and is peculiarly disposed, giving off a very small branch on the right side about the middle of its length.

Acknowledgments

The present work was carried out under the direction of my teacher Mr. Beni Charan Mahendra, to whose continuous assistance and painstaking criticisms I owe a great deal. I am also indebted to the authorities of St. John's College, Agra, for the facilities enjoyed by me in the Zoological Laboratory.

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A



B

The abnormal *H. flavoviridis* Bluppel
(A) Dorsal View (B) Ventral View

PHOTOPERIODISM AND THE PHASIC DEVELOPMENT OF *CROTALARIA JUNCEA*

BY B N SINGH AND S N SINGH

(From the Institute of Agricultural Research, Benares Hindu University)

Received December 2, 1938

Introduction

FROM a previous contribution,¹⁸ dealing with the influence of photoperiodism on the manurial efficiency and the distribution of *Crotalaria juncea* it is evinced that the length of the exposure induces characteristic variations in the plant development. A good deal of literature on this subject both of scientific and economic importance has already been accumulated. Adams,¹⁻⁵ Tincker,¹⁹⁻²¹ Reid,¹⁵ Gilbert,^{12,13} Nightingale,¹⁴ and Garner and Allard⁶⁻¹¹ investigated the response made by plants to altered conditions of illumination. Although the accumulated data are sufficiently comprehensive, they fail to elucidate in its entirety the causal relationship between plant growth and varied conditions of illumination, a phenomenon which necessitates the study of internal conditions of a plant varyingly illuminated.

The present contribution aims at the elaboration of this aspect with special reference to the relationship between photoperiodism and the phasic development of *Crotalaria juncea*. Observations in the variations induced in the H-ion concentration and the C/N ratio of the exposed tissue have been recorded, and the probable explanation for the specific changes in the plant advanced.

Methods and Material

The hours of illumination were arranged as follows

	Exposures		Time
Sub-normal	1 hour		12 NOON to 1 P.M.
	3 hours	. .	11 A.M. to 2 P.M.
	6 hours	. .	10 A.M. to 4 P.M.
	8 hours	.. .	9 A.M. to 5 P.M.
Normal	11 hours		7 A.M. to 6 P.M.
Supra-normal	16 hours	. .	7 A.M. to 11 P.M.
	20 hours	.. .	7 A.M. to 3 P.M.
	24 hours	. .	Continuous

The rest of the experimental procedure has already been detailed in the previous contribution.¹⁸ Growth was measured in terms of dry matter

production and the shoot-root ratio was calculated on dry weight basis. The methods for the estimation of total carbohydrates and nitrogen for computing C/N ratio were the same as given elsewhere¹⁷. The H-ion concentration was determined electrometrically with calomel quinhydrone electrode.⁷

Summary of Results

General Characteristics—Soon after the exposure to varied durations of light, the plants began to show characteristic variations. The number of flowers set under 20 hours exposure was only 39 as against 64 under 16 hours illumination. Under continued illumination the plant restricted its growth to the purely vegetative phase. The number of days elapsing from germination to the appearance of first blossoms also shifted in close agreement with the increase in the period of diurnal illumination.¹⁸

Dry Matter Production—With an increase in the daily illuminating hours there is a concomitant increase in the dry matter production. When the dry weights are plotted against age, the time-weight curves are found to be identical (Fig. 1) with the typical sigmoid curve of the mono-molecular autocatalytic reaction only in case of the plants exposed to normal

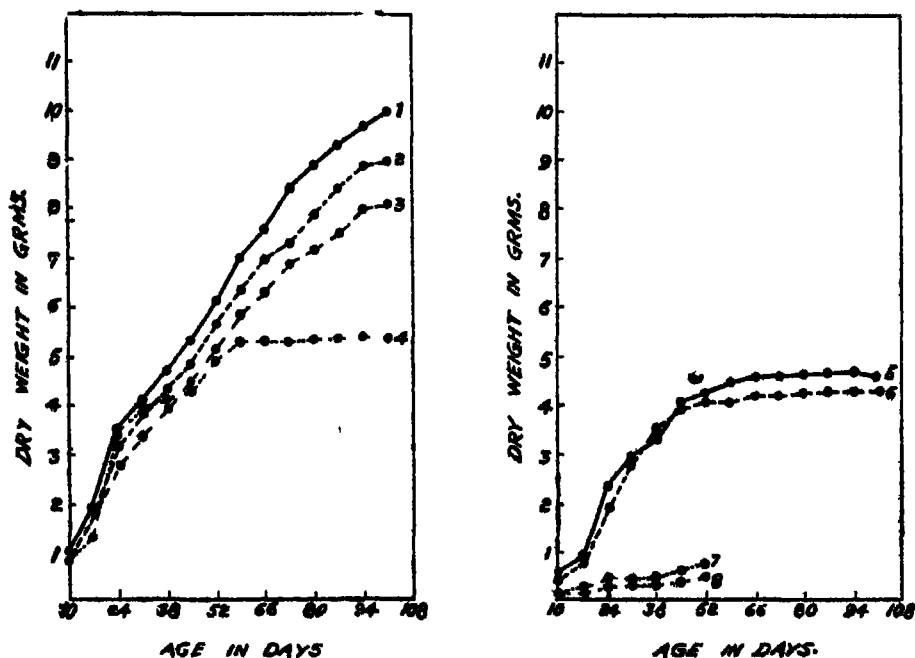


FIG. 1

Curves 1-8 representing dry matter accumulation in grammes per plant in *Crotalaria juncea* under the influence of 24, 20, 16, 11, 8, 6, 3 and 1 hours daily exposures respectively

day length The curves for six and eight hours exposures are also similar but the rest both for sub-normal as well as supra-normal do not show a strict resemblance The former are extremely flat whereas the latter are strikingly straight A change in the light duration induces a change in the form of the dry weight curves As the day length increases to supra-normal conditions the point of inflexion of the time-weight curves is progressively delayed, with the gradual disappearance of the autostatic phase If according to the conception of Robertson and others¹⁶ growth were indeed a simple autocatalytic process, the alteration in the range of illumination would have changed the rate of the entire process and could not have conceivably altered the form of the time-weight curve

TABLE I

*Variations induced in the shoot-root ratio of Crotalaria juncea
as a result of varying illuminational hours*

Age in days	Various light durations (in hours)							
	1	3	6	8	11	16	20	24
10	1.80	1.30	2.00	1.95	1.70	1.71	2.03	2.00
17	1.91	1.80	2.80	2.10	2.20	2.08	2.10	2.16
24	2.10	2.40	5.41	6.60	7.30	3.40	4.10	4.58
31	1.87	1.90	6.60	6.64	7.20	3.90	4.80	4.94
38	1.72	1.60	7.58	6.90	4.90	4.69	5.20	5.82
45	1.32	1.40	4.60	4.40	4.90	4.30	4.70	3.50
52			4.20	4.40	5.10	3.70	4.20	3.00
59			4.06	4.41	4.90	3.80	3.80	2.80
66			4.06	4.34	4.30	3.20	3.00	2.60
73			3.80	4.20	3.40	2.80	2.70	2.16
80	.		3.70	3.70	3.43	2.40	2.43	1.90
87	.	.	3.10	3.02	3.23	1.80	1.90	1.70
94	..	..	2.09	1.47	1.70	1.20	1.30	1.36
101	.	..	2.01	1.10	1.60	1.11	1.01	1.21

Shoot/Root Ratio—Under normal light conditions this ratio ranges between 1.7 to 7.3 (Table I), the highest being attained 24 days after

germination. Subsequently the ratio declines, reaching the minimum value at the senescent stage. The ratio is in inverse proportion to the length of the exposure, in other words the duration of illumination seems to have a positive correlation with the root development, be it a result of the negatively geotropic nature of the roots or the increased availability of the translocated food material in response to the accelerated photosynthetic efficiency of the plant.

H-ion Concentration—The pH value of the plant sap, extracted from the growing parts of the stems including a number of young leaves, of the various experimental sets also appears to be influenced by the length of the day (Table II). Under shorter durations of illumination the fluctuations,

TABLE II

Variations induced in the pH of the cell sap of Crotalaria juncea as a result of varying illuminational hours

Age in days	Various light durations (in hours)							
	1	3	6	8	11	16	20	24
10	5.02	6.00	5.20	4.80	4.40		4.10	4.82
17	5.20	4.90	4.80			4.81	4.87	4.64
24	5.80	5.90	6.02	6.01	5.42	5.52	5.92	5.43
31	7.09	6.43	6.40	5.60	5.60	5.51	5.33	5.28
38	6.91	6.50	5.30	5.25	5.82	5.22	5.21	5.20
45	6.50	6.40	5.20	5.16	5.29	5.41	5.42	5.07
52			5.26	5.20	5.31	5.42	5.33	5.02
59			5.18	5.70	5.80	5.91	5.61	5.08
66			5.68	5.80	5.90	5.81	5.70	5.07
73			5.97	5.90	5.91	5.83	5.81	5.07
80			6.12	5.80	5.85	5.91	5.72	5.06
87		.	6.40	5.60	5.70	5.82	5.74	5.11
94		.	6.70	5.65	5.60	5.62	5.92	5.09
101	.	.	5.40	5.59	5.40	5.33	5.80	5.09

specially during the early stages are more pronounced than those under supra-normal conditions of illumination. Towards the later stages of the

life-cycle the pH values under eight and eleven hours run more or less concurrently whereas those under six hours show a rising tendency.

The plants grown under continued illumination, however, maintain a less fluctuating and more or less even value for the major portion of the life-cycle. When compared to the values under other sets, acidity under continued illumination appears to be maximum specially during the period when the plants of other series have initiated the development of the reproductive organs. It is interesting to note that after exposure of plants growing under continued illumination, to darkness a decrease in acidity at the growing points is observed with the simultaneous development of the rudimentary buds (Table III)

TABLE III

The influence of darkness on the pH value of the sap of growing points of Crotalaria juncea, grown under continued illumination

Samples	pH value				
	Before keeping in darkness	After 24 hrs	After 36 hrs	After 48 hrs	When buds appeared
1	5.09	5.32	5.61	5.82	5.71
2	5.01	5.22	5.71	5.80	5.68
3	5.03	5.40	5.63	5.91	5.73

Carbohydrate/Nitrogen Ratio—In plants exposed to full day length (11 hours) the ratio fluctuates between 2.86 to 6.52 (Table IV). Under six, eight and eleven hours durations with an increase in the age of the plant C/N ratio also appears to show a parallel increase. This increase of the ratio during the later stages is the result of the high carbohydrate content of the plant. With increasing exposures the range of fluctuations becomes narrower, and under continued illumination an even value is maintained throughout the entire life-cycle of the plant. Supra-normal exposures bring about an increase in both, the carbohydrate content through increased photosynthetic efficiency and nitrogen content through increased absorption by extensive underground plant parts. The result is that the ratio does not fluctuate over wider limits. A simultaneous accumulation of these constituents as has been shown in the previous article¹⁰ may probably account for the low C/N ratio in whole day (24 hours) exposed plants and consequently continued vegetative growth.

TABLE IV

Variations induced in the C/N ratio of Crotalaria juncea as a result of varying illuminational hours

Age in days	Various light durations (in hours)							
	1	3	6	8	11	16	20	24
10	5.08	2.84	2.85	2.71	2.94	2.99	2.59	3.31
17	4.42	3.43	3.18	3.17	3.32	3.38	2.88	3.09
24	3.27	4.43	3.08	2.90	2.86	3.08	3.00	2.87
31	2.82	2.90	2.72	2.83	3.09	3.70	3.62	3.43
38	2.79	3.71	4.03	4.27	3.90	4.73	5.03	3.13
45	3.41	3.95	4.80	5.02	4.90	4.80	5.27	3.23
52			5.24	4.84	4.19	4.70	5.16	3.40
59			5.20	4.70	3.55	3.90	5.06	3.34
66			6.48	4.22	3.63	3.56	4.29	3.40
73			7.18	4.45	3.80	3.49	3.42	3.56
80			7.46	4.87	5.80	4.06	3.26	3.25
87			7.93	4.36	4.10	3.90	3.46	2.84
94			8.10	4.02	5.76	4.10	3.38	2.78
101			7.85	5.45	6.32		3.10	3.03

Discussion

From the foregoing results it is apparent that the duration of illumination has a remarkable regulatory action on the phasic development of the plant. Plants attain flowering and fruiting only when the period of illumination falls within a minimum and maximum range of diurnal illumination. When it is increased beyond the maximum limit the vegetative development continues at the cost of sexual or otherwise. When the length of the exposure lies between these two limits both vegetative and reproductive forms of activities progress simultaneously and the plants appear to be normal in habit.

Garner and Allard opine that the highly refined regulation of the degree of protoplasmic hydration furnishes a basis for the various plant responses to changes in light period. According to Blackman⁸ the photoperiodic reaction is a complex one and is exceedingly difficult to be interpreted in

terms of physiology. Indeed it is far from arriving at a particular conclusion about the nature of the reaction but on the basis of the present observations these changes may be associated with the accumulation of the plant food materials, the concentration of which seems to have a regulatory action on the phasic development of a plant. Under high light durations the accumulation of carbohydrates and nitrogen compounds takes place at a much faster rate in consequence of which the C/N ratio, which has been found to play a dominant role in the initiation of reproductive primordia, is considerably lowered. Moreover the production of the toxic acids is a characteristic feature during the synthesis of carbohydrates and proteins. These acids have been found to delay flowering considerably. Under shorter exposures as a result of low nitrogen content the high C/N ratio results in an early appearance of flowers.

Further when the plants growing under continued illumination are transferred to complete darkness, the fall in the pH value of the cell sap of the growing points is suggestive of the fact that such a change in the environment of the plant offers a check to the further accumulation of the metabolites and those already accumulated are utilized as a source of energy in the downgrade processes, which ultimately results in the appearance of the buds. Further as the light duration is shortened the amount of photosynthetic products falls, whereby the condition favourable for flowering with regard to C/N ratio as well as specific acidity were brought about much earlier, with the net result that the autokinetic phase of the growth curve is shortened and soon followed by the autostatic one. Thus the photoperiodic response of a plant may be based upon the fact that the absorption,¹⁸ synthesis and the accumulation of the protoplasmic ingredients is different under varying exposures, and it is the concentration of these metabolites of whatever nature which seems to form a weak-link in the chain of the physiological activities of the plant accompanying the transition from the vegetative to the reproductive stage, ultimately changing the form of the time-weight curve.

Conclusions

As the duration of light is increased above the normal the concentration of the total carbohydrates and nitrogen increases resulting in a diminished range of C/N ratio. A concomitant increase in the acidity value of the plant sap is observed with the length of the day. Accumulation of such metabolites appears to play an increasingly significant role in governing the initiation of the reproductive phase, in modifying the nature of the growth curve and thus inducing abnormalities in the organism.

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THE INFLUENCE OF MOISTURE CONTENT AND NITRATE FEEDING ON THE NITROGEN CYCLE IN THE SOIL BOTH UNDER AEROBIC AND ANAEROBIC CONDITIONS

BY B N SINGH, S N SINGH

AND

K M NAIR

(From the Institute of Agricultural Research, Benares Hindu University)

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Introduction

THE availability of nitrogen in the soil and its continuous maintenance has been one of the most baffling problems confronting the agriculturist. Soils at present are no more looked upon as dead reservoirs of plant food but as substrates teeming with organised life. In contrast to the extreme chemical view formerly held, the soil organisms are now considered to be of more fundamental importance in the decomposition of practically all organic substances.

Of the various soil processes determining the productivity of the soil ammonification and nitrification are considered to be of great significance. Liebig in 1840 considered that the process of nitrification was one of slow oxidation of organic nitrogen and named it "Eremacausis".¹ In 1862, Pasteur expressed the view that nitrification in soils might be a biological process which was later confirmed by Schlosing and Muntz² in 1877. The biological nature of nitrification having thus been established numerous investigations were made later to isolate the organisms concerned in the various phases of this complex dynamic phenomenon beginning from the organic nitrogenous material as such right up to the formation of nitrates. Winogradsky in 1890 by employing a medium composed of silica jelly supplied with inorganic nutrients successfully isolated the organisms and facilitated the study of nitrification in pure culture apart from soil as a medium.

Formerly much emphasis was placed on the number of the soil micro-organisms but it is only later that their physiological efficiency has been recognised as a conditioning factor. As soil nitrogen is mostly derived from vegetable proteins, its chemistry consists of their changes proceeding under

complex and variable conditions. The environmental complex under which these changes are proceeding not only determine the velocity of the phenomenon but also the final products of the reaction. Of these variables soil moisture, soil aeration, and the nutritional balance of the soil undoubtedly play an increasingly significant rôle in determining the rate of the two processes.

This, in brief, was the outlook with which the present series of investigations were instituted to study the influence of varying percentages of moisture content on the processes of ammonification and nitrification. The influence of increasing percentages of nitrates on the process of ammonification both in soil and pure cultures, under different conditions of moisture content and aeration, was also studied. It was further arranged, to study the true behaviour of ammonifiers and the cause of the disappearance of nitrates in pure culture.

Experimental Plan

The experiments were grouped under the following heads

- A The validity of the "proposed method" for measuring ammonification
- B The influence of various percentages of moisture content on both ammonification and nitrification
- C The influence of nitrates on ammonification under the following conditions
 - (a) Aerobic and optimum water content
 - (b) Anaerobic and optimum water content
 - (c) Water-logged, with super-incumbent air
 - (d) Water-logged, without super-incumbent air
 - (e) Anaerobic condition in pure culture

Experimental

The soil under investigations was loam in nature and taken from the upper layers of the farms attached to this Research Station. It was air-dried to constant weight, crumbled well with the hand to pass through a 2 mm sieve. The sifted soil was thoroughly mixed in order to make it a composite mass.

The tumbler method after Lipman^{1,2} was followed in the groups of the experiments coming under the heads A, B, C (a) and C (e), mentioned above. In C (b) and C (d) wide-mouthed bottles with air-tight corks having an exit tube to exhaust the air and thus ensure anaerobic conditions were

used In the case of C (e) conical flasks with air-tight corks and with exit tubes to exhaust air were employed These apparatus were all sterilized in an autoclave at a pressure of 20 lbs per square inch for experimentation

100 gm of air-dried soil were put in the tumblers 2 gm of sterilized dried blood containing 15.8 per cent of organic nitrogen were added with a sterile spatula and mixed well with the soil The tumblers under experiment A were arranged in eight replicates, four for estimating the ammonification and four for estimating the nitrate content As for head B the tumblers were arranged in eleven colonies each with a specific moisture content of the soil which was maintained throughout the period of incubation Heads C (a) and C (b) contain five colonies supplied with various percentages of sodium nitrate at a range of 0.0 – 0.5 per cent at 0.1 per cent intervals and optimum quantity of water In case of heads C (c) and C (d) five sets of the experiments come under each head, with water-logged soil and the percentages of nitrates at the ranges and intervals already cited above The experiments coming under C (c) were carried out in conical flasks containing 50 c.c. of a 2 per cent solution of peptone into which equal numbers of ammonifying organisms were introduced by inoculating aliquot parts from a pure culture reared in a medium, composed of peptone, potassium acid phosphate, agar, and tap water¹⁰ and diluted in sterilized distilled water to form a uniform suspension

The soils as well as cultures were incubated at a temperature between 26° and 31° C The period of incubation in all cases was six days except in B where it was extended to 14 days to facilitate the study of nitrification also

Methods for the Estimation of Ammonification—The estimation of ammonification of organic nitrogenous compounds applied to soils is so far carried out by absorbing the escaping ammonia in acids of known strength after expelling it by boiling with magnesia or any other strong non-volatile alkali.¹ The process does not seem to be free from difficulties, firstly, in the sense that ammonia which undergoes volatilisation during the incubation period, is not taken into consideration Secondly, to derive an appreciable result the period of incubation for ammonification must be fixed to one of very short duration, else practically no ammonification could be observed due to the continuous conversion of ammonia to nitrites and nitrates Even if the period of incubation be one of very short duration a certain quantity of the ammonia produced is definitely converted to nitrates and as such by this method it is far from being a correct estimation of the

absolute ammonification There is yet another anomaly in the method and that is, due to the different arbitrary periods so far employed in each case no correlation between the velocity of the two processes, *viz*, ammonification and nitrification can be established.

The process of ammonification is the result of the hydrolysis of the organic nitrogenous material Thus a part of the original organic nitrogen in the soil is converted into mineral nitrogen, either existing in the soil as ammonia or as ammonia volatilised or ammonia which is converted to nitrates It is, therefore, obvious that for studying ammonification all these fractions of ammonia must together be taken into consideration Moreover, the nitrates under anaerobic conditions may also be reduced to nitrites, free nitrogen or ammonia thus interfering in the estimation of ammonification The difficulties thus mentioned invalidate the direct estimation of ammonia present in the soil as an index of ammonification of the organic nitrogenous material added to the soil nor can a combined direct estimation of ammonia and nitric nitrogen enable one to surmount the difficulties caused by denitrification To avoid these errors the criterion for the observation of absolute ammonification may be based upon the examination of the total organic nitrogen in the soil both before and after the period of incubation The difference gives the amount of organic nitrogen converted to ammonia, be it existing in any form whatever The non-symbiotic fixation of free atmospheric nitrogen also cannot interfere with the accuracy of this method since according to Bonazzi,⁷ it seldom occurs when the soil is richly supplied with nitrogenous material

Proposed Method for Estimating Ammonification—A weighed quantity of soil sample was taken and the ammonia contained therein was expelled by boiling with magnesia The residue was air-dried, powdered and employed for estimation of total nitrogen including nitric nitrogen⁸ From another duplicate of the same experiment nitric nitrogen was determined according to the method followed by Greaves and Carter⁸ The difference between the two estimations gave the total organic nitrogen present in the soil The same procedure was followed both before and after the incubation period and the difference in the two estimations of organic nitrogen was taken to be the measure of ammonification The comparative values for ammonification in similar samples as obtained by the two methods are given in Table I

It is evident that in all the four separate estimations the percentage of nitrogen estimated as ammonification by the "Proposed method" is always higher than that obtained by the direct estimation of ammonia by boiling

TABLE I
Comparison of the Methods for Estimation of Ammonification
(Mgm of nitrogen per 100 gm of the soil)

Experiment	Organic nitrogen before experiment	Organic nitrogen after experiment	Ammonified organic N ₂ by proposed method	Ammonia expelled by ammonia + NO ₃ N	Difference
1	344.96	291.20	53.76	49.43	4.34
2	..	292.60	52.36	49.56	2.80
3	..	291.90	53.00	48.42	4.64
4	..	292.46	52.50	50.62	1.88

with magnesia The difference between the two estimations seems to be quite significant It ranges between 1.88 and 4.64 mgm of nitrogen

Experimental Findings

With an increase in the percentage of moisture content of the soil an increase in both ammonification and nitrification is observed till 51 per cent and 34 per cent of the moisture holding capacity of the soil respectively

TABLE II
Influence of Moisture Content on Ammonification and Nitrification
(Mgm. of nitrogen per 100 gm of the soil)

Experiment	Water content in percentage of the moisture holding capacity of soil	Nitric nitrogen	Ammonified organic nitrogen
1	0.0	13.30	14.42
2	17.0	23.80	29.26
3	34.0	53.90	60.76
4	51.0	51.10	63.14
5	68.0	35.00	50.26
6	85.0	25.20	44.40
7	102.0	12.60	35.86
8	119.0	5.16	29.40
9	136.0	0.00	25.90
10	153.0	0.00	24.22
11	170.0	0.00	24.50

The minimum value for ammonification is 14.42 mgm. in air-dried soil, whereas for nitrification the minimum value is obtained under over-saturated conditions. In the case of nitrification even the complete absence of water does not show such an inhibiting effect as saturation. The reverse is true for ammonification. With an increase in moisture content a parallel increase in both ammonification and nitrification is noted reaching the maxima under 51 per cent and 34 per cent respectively. Further increase in the water content resulted in a continuous fall with the difference that the degree of retardation varies in the two cases, nitrification falling at a rapid rate till 119 per cent moisture content beyond which the bacterial activity seems to be completely arrested. On the other hand, ammonification decreases but only gradually and under 136-170 per cent of the moisture holding capacity of the soil, the values fluctuate within a very narrow range. Especially in the last two cases ammonification is more or less constant. It denotes that even under water-logged conditions the ammonifiers maintain their efficiency and continue to bring about the biological changes in the soil. It is also remarkable to note that both the processes seem to be more or less equally influenced by the moisture content so far as the maxima are concerned. It may be due to the fact that nitrification is preceded by ammonification and an increase in the rate of the latter is liable to result in an increased acceleration of the dependent phenomenon.

Coming to the other aspect of feeding the bacteria with nitrates under various conditions of aeration and moisture content it is seen that under

TABLE III

Influence of Sodium Nitrate and Optimum Water Content (51 per cent of the Moisture-holding Capacity of the Soil) and Aerobic Conditions on Ammonification

(Mgm of N_2 per 100 gm of soil)

Experiment	% of sodium nitrate added	Organic nitrogen before experiment	Organic N_2 after experiment	Ammonified organic N_2	Difference between final and initial $NO_3 \cdot N$ content
1	0.0	344.96	298.34	46.62	+ 2.38
2	0.1	„	282.52	62.44	+ 2.87
3	0.2	„	272.16	72.80	+ 3.08
4	0.3	„	289.66	55.30	- 1.47
5	0.4	„	292.60	52.36	- 2.87

TABLE IV
Influence of Sodium Nitrate under Optimum Water Content and Anaerobic Conditions on Ammonification
(Mgm. of nitrogen per 100 gm. of soil)

Experiment	% of sodium nitrate	Organic nitrogen before experiment	Organic nitrogen after experiment	Ammonified organic nitrogen	Difference between final and initial $\text{NO}_3\text{-N}$
1	0 0	344 96	333 62	11 34	- 1 96
2	0 1	„	313 54	21 42	- 2 52
3	0 2	„	314 72	30 24	- 2 66
4	0 3	„	321 30	23 06	- 4 62
5	0 4	„	325 30	19 60	- 7 00

TABLE V
Influence of Sodium Nitrate under Water-logged Condition with Super-incumbent Air
(Mgm. of nitrogen per 100 gm. of soil)

Experiment	% of sodium nitrate	Organic nitrogen before experiment	Organic nitrogen after experiment	Ammonified organic nitrogen	Difference between final and initial $\text{NO}_3\text{-N}$
1	0 0	344 96	332 36	12 60	- 1-40
2	0 1	„	323 40	21 56	- 3 00
3	0 2	„	317 66	27 30	- 4 06
4	0 3	„	310 56	34 40	- 5 88
5	0 4	„	302 96	42 00	- 7 49

TABLE VI
Influence of Sodium Nitrate under Water-logged Condition without Super-incumbent Air
(Mgm. of nitrogen per 100 gm. of soil)

Experiment	% of sodium nitrate added	Organic nitrogen before experiment	Organic nitrogen after experiment	Ammonified organic nitrogen	Difference between final and initial $\text{NO}_3\text{-N}$
1	0 0	344.96	335 30	9 66	- 2 80
2	0.1	„	334 46	10 50	- 3 64
3	0.2	„	330 26	14.70	- 4 83
4	0 3	„	325 22	19.74	- 6.44
5	0.4	„	318.08	26.88	- 8 12

aerobic as well as anaerobic conditions with optimum water content nitrates are found to stimulate the process of ammonification till a particular concentration of nitrates is reached in the soil (Tables III and IV) The optimum results for ammonification are obtained under 0.2 per cent of nitrate feeding Further increase in the nitrate percentage inhibited to a marked degree the activity of the ammonifiers The ammonification values for the experiments C (a) and C (c) are proportionately higher than those obtained for C (b) and C (d) Under water-logged conditions, ammonification increases gradually and the maximum is attained under 0.4 per cent concentration of nitrates (Tables V and VI) The difference in the behaviour of nitrates under optimum water and water-logged conditions is very patent for the fact that in the former the nitrate optimum is 0.2 per cent while in the latter it is 0.4 per cent or even above This difference may be attributed to the varying percentages of moisture content Under water-logged conditions even after the supply of maximum dose of nitrates the concentration of this nutrient in the liquid phase appears to remain so low as not to become toxic as observed under the other set The high concentrations of the nitrates seem to check the activity of ammonifiers

Under optimum water content and anaerobic conditions, in the last two cases there is a fall in the ammonification values and the corresponding loss of nitrates is more It is probable that till 0.2 per cent concentration of sodium nitrate, the ammonifiers are highly active and supersede an entirely different group of organisms known as denitrifiers The latter concentrations appear to stimulate the denitrifying organisms and partly maintain the activity of the ammonifiers also As a result of this the loss of nitrate continues at a faster rate with lesser ammonification

Under water-logged condition with super-incumbent air (Table V) ammonification is much more increased than what it is in the complete absence of it (Table VI) The difference is evidently due to the non-aerated conditions of the latter. An increased quantity of nitrates here also seems to contribute significantly to the ammonifying power It appears that under such conditions both the nitrates and the super-incumbent aeration influence the process simultaneously

The previous observations on the soil cultures were further confirmed by employing pure cultures of ammonifiers It is observed that the presence of nitrates accelerates ammonification and brings about a rapid decomposition of peptone under anaerobic conditions.

TABLE VII

Ammonification in Peptone Medium under Anaerobic Conditions

Experiment	% of Nitrate added	Equivalent in nitric nitrogen in mgm	Nitric nitrogen recovered after incubation in mgm	Gain or loss in mgm	Ammonified nitrogen in mgm
1	0.0	0.0	0.0	0.0	Nil
2	0.1	14.7	12.95	- 1.75	60.27
3	0.2	29.4	26.95	- 2.45	72.66
4	0.3	44.1	43.05	- 1.05	50.12
5	0.4	52.8	57.82	- 0.98	46.20

In control culture experiments in the absence of nitrates no ammonification could be observed but the increased concentration, viz, 0.0-0.2 per cent of the salt, stimulated the breakdown of peptone. Further increase beyond the maximum brings about a retardation in the activity of the ammonifying organisms but the loss of nitrates, observed for this retarded ammonification power, is correspondingly low.

Discussion

From the foregoing observations it is obvious that the optimum water required for ammonification under aerobic conditions is 51 per cent and that for nitrification is 34 per cent of the moisture holding capacity of the soil. According to Versulys⁴ with increasing moisture content the cellular pores of the soil assume first a "pendular stage". Then due to increasing pressure of the entering water the air in the spaces assume a "funicular" form, when the air as well as water become continuous between pore and pore. The pendular stage exists from 6 per cent of water filling the porous space to 24 per cent, while the funicular stage can exist side by side with the pendular, for the same range of moisture content and even a little above the final percentage. From the data in Table II it is evident that the optimal water for ammonification and nitrification lie within those ranges of soil moisture which do not exclude soil air to a considerable extent. At those stages the air within the soil pores exists within the soil either in the pendular form or the funicular form, while under higher soil moisture the retarded bacterial activity is the result of poor aeration.

Coming to the other aspect it is seen that the addition of nitrates introduces variations in the ammonification values of the variously treated sets. The loss of nitrates and the corresponding values for ammonification though

not always proportionate to each other may, however, find an explanation. One may, though it is not exactly the fact, conclude that the increase in ammonification is due to the corresponding decrease in nitrates supplied to the soil, but the criterion of ammonification in these experiments as carried out according to the "proposed method" has been the difference between the total organic nitrogen before and after the period of incubation and as such the products of denitrification can in no way affect the values obtained for ammonification. Thus the loss of nitrates even if it be entirely through denitrification is not contributive to the increases in the ammonification value which is independent of the loss of nitrates.

Moreover, a display of increased activity of the ammonifying organisms shows a corresponding loss of nitrates within certain limits in soil cultures. The fact that for the higher percentages of the salt, excepting in water-logged conditions where its concentration has not reached the optimum, the results yielded for ammonification are comparatively low and the corresponding loss of nitrates is heavy beyond proportion. The low results for ammonification is due to the toxic effect of high concentration of sodium nitrate on the ammonifying organisms and the heavy loss of nitrate may be due to the vigorous setting in of denitrification.

Another interesting feature observed lies in the respective higher values for ammonification obtained under aerobic condition. The influencing factors under such a condition are both aeration and nitrate supply and as such these variations may be attributed either to the existence of two different groups of ammonifiers, one aerobic and another anaerobic in nature, or to the facultative anaerobism exhibited by ammonifiers. In the first instance the observed increases in ammonification under aerobic conditions may be explained, as being due to the combined functioning of the specific groups because the soil is well aerated and as well supplied with oxygen in the form of nitrates. If the second view, that the ammonifiers depend entirely on their facultative anaerobic capacity for bringing about ammonification under anaerobic conditions, be adopted, then the decreased ammonification under anaerobic condition may very well be attributed to a limited source of oxygen supply in the form of nitrates only the higher doses of which seem intolerable to the ammonifiers.

In pure cultures we exclude all the organisms except the ammonifiers and as such any loss of nitrate must be due to the ammonifiers alone. Moreover in this case with higher concentrations of nitrates, though the ammonification is low it is proportional to the loss of nitrates, showing thereby that there is a positive correlation between nitrate consumption and the

ammonifying power The significance of such an inference may be based upon the supply of energy to the ammonifiers through nitrate feeding, be it a group of aerobic or anaerobic organisms or the ammonifiers as a whole exhibiting facultative anaerobism

Summary and Conclusions

The paper deals with the influence of moisture content on ammonification and nitrification, and of nitrate feeding conditions of moisture and aeration on ammonification

A method is proposed for measuring ammonification The proposed method for measuring ammonification based upon the difference in organic nitrogen both before and after the incubation period has been found to give better results for ammonification

There is a particular range of moisture content, both for optimum ammonification and nitrification, being between 34 and 51 per cent of the moisture-holding capacity of the soil

Under saturated conditions nitrification is completely inhibited whereas ammonification continues though at a slower rate

Nitrates influence the ammonifying organisms to increased activity till a particular concentration is reached beyond which the process is retarded

The retardation of ammonification is accompanied by setting in of denitrification

The ammonifiers seems to be composed of two groups, one aerobic and the other anaerobic in nature, if aerobic they exhibit facultative anaerobism under unfavourable conditions

Under aerobic conditions the activity of the ammonifying organisms is proportionately higher

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OBSERVATIONS ON BREEDING IN BRACKISH-WATER ANIMALS OF MADRAS

BY N. KESAVA PANIKKAR AND R. GOPALA AIYAR
(From the Department of Zoology, University of Madras)

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Introduction

IN a previous issue of these *Proceedings*, we gave a general account of the brackish-water fauna of Madras in which the general trend in the breeding habits of the majority of the species observed by us was indicated in a brief summary (Panikkar and Aiyar, 1937). The following account is a continuation of the same topic that was but lightly treated in that paper. We submit here the biological notes with special reference to breeding in the more common species of brackish-water organisms of Adyar. The period of observation extended to a little over three years. These records are by no means exhaustive, and, having not made intensive studies on every species encountered, all that is attempted by us in this paper is to give a preliminary account of the reproductive periods of some of the very common forms in so far as we have been able to observe them during the period of our faunistic survey. We have thought this account worth while in view of the growing importance of the subject of breeding in tropical marine animals and of the fact that, in some of the species noted, we have new information on their biology.

The criteria on which the active spawning seasons have been determined are different for the different species, depending upon the spawning habits of the organisms in question. Detailed examination of gonads of specimens collected during different months has been made in the case of Anemones, and to a certain extent in the bivalves and the Polychaetes. The presence or absence of ovigerous females formed the main criterion for judging the breeding seasons of Decapods (excepting the Penaeidae), while in the case of

certain Gastropods, the occurrence of spawn masses was mainly relied upon. The presence of embryos in the brood pouches provided the necessary information so far as Amphipods, Schizopods and Isopods were concerned. These observations were made whenever specimens were collected from the backwater or the river at Adyar and from Cooum. Plankton collections from the Adyar backwater and the river have appreciably supplemented these observations. The general conclusions that may be arrived at from these records are summarised in this paper. There is not the least doubt that a considerable amount of variation does take place depending upon variations in climatic changes in successive years (as for example, the beginning of the Monsoon) and no study of breeding seasons may be considered complete unless observations have been continued for a number of years. Further studies carried out for a much longer period than the present one may be expected to bring further corrective data bearing on the subject, but the purpose of this paper is to indicate the breeding seasons in broad outlines which in itself may facilitate a later detailed study.

Environment

For a detailed description of the brackish-water areas of the City of Madras reference may be made to our previous paper. In addition to the information given there, we give below the tables showing the monthly rainfall at Madras for the years during which these observations were made, because there is reason to believe that rainfall plays a significant rôle in the habits of many of the organisms that we have observed in the peculiar brackish-water habitats.

Tables showing monthly rainfall at Madras

Month				Total rainfall for the month (Inches)	Departure from normal
1933					
January	..	..	..	0.00	- 1.39
February	.	..	.	0.00	- 0.32
March	.	..	..	3.42	3.23
April	..	..	..	0.03	- 0.50
May	..	..	..	0.07	- 1.00
June	..	..	..	0.75	- 1.14
July	..	..	..	1.08	- 2.86
August	..	.	..	3.00	- 1.64
September	..	..	..	1.20	- 3.79
October	..	..	..	9.87	- 1.65
November	..	..	.	5.76	- 8.49
December	..	..	..	15.00	9.19

Month				Total rainfall for the month (Inches)	Departure from normal
1934					
January	..	.	.	2 05	0 66
February	.		.	0 00	- 0 32
March		..	.	0 00	- 0 19
April	..	.	.	0 74	0 21
May	..	.	.	0 00	- 1 07
June	.	.	.	1 76	- 0 13
July	.	.	.	2 07	- 1 87
August	.	.	.	7 17	2 53
September	..	..	.	2 00	- 2 99
October	.	.	.	16 82	5 10
November	.		.	1 86	- 12 39
December			.	2 19	- 3 62
1935					
January	.		.	0 57	- 0 82
February		.	.	0 00	- 0 32
March			.	0 00	- 0 19
April	.	.	.	0 00	- 0 53
May	.		.	0 00	- 1 07
June		.	.	1 23	- 0 66
July	.	.	.	2 21	- 1 73
August			.	9 07	4 43
September	.		.	2 98	- 2 01
October	.	.	.	15 53	3 81
November	.		.	6 40	- 7 85
December	.		.	2 35	- 3 46
1936					
January	..	..	.	0 10	- 1 29
February	.	.	.	2 53	2 21
March	.		.	0 90	0 71
April	..	.	.	0 46	- 0 07
May	.	.	.	0 02	- 1 05
June	..	.	..	2 82	0 93
July		.	.	4 87	0 93
August	..	.	.	5 99	1 35
September	..	.	..	1 93	- 3 06
October	..	..	..	8 16	- 3 56
November	..	..	..	14 53	0 28
December	..	..	.	1 94	- 3 87

*Observations on Breeding*PHYLUM . *Cœlenterata*CLASS . *Hydrozoa**Campanulina ceylonensis* (Browne)

This is one of the Hydrozoans that may be considered as a permanent inhabitant of the brackish-waters of Madras, the species is also common on the Madras coast, Chilka Lake and Gangetic delta. The medusæ are frequently observed in the backwaters at Adyar, pools adjoining the mud-flats near the Elphinstone Bridge, the Cooum, and in certain seasons even in the Buckingham Canal. Though found throughout the year as evidenced by our plankton records, the specimens were obtained in large numbers only from June to October. Most of the individuals collected during this period had well-developed gonads and ripe germ cells. October to February collections of the plankton showed that their numbers considerably fall during these months, but ripe individuals were observed even during this period. The medusæ collected during the summer months from March to June were not in a perfectly healthy condition since most of them were without tentacles, and all the specimens collected during March-May period of 1934 and March-June period in 1935 were without well-developed gonads and ripe germ cells. We have not so far discovered the Hydroid generation of this species in the locality studied. Annandale (1922) reports that the Hydroid which is very minute and found attached to bricks and rocks is an exceedingly delicate organism unlike the medusa which in spite of a somewhat fragile appearance is extraordinarily hardy. From our records it appears that the sexual reproduction of the medusæ takes place from June to February, showing a great intensity during the July-September period. The asexual reproductive activity of the Hydroids also seems to be well marked during June-August. Large numbers of young ones of the medusoid generation appears a day or two after the few showers at the end of summer.

Dicyclocoryne filamentata (Annandale)

As in the previous species only the medusoid generation has been discovered by us. The species is rather rare and specimens have been collected by us only during August 1936. But the fact that very young medusæ have been collected during this period suggests that asexual budding of the Hydroid must have been taking place at this period.

Campanularia noliiformis McCrady*Laomedea (Obelia) spinulosa* Bale

Only the Hydroid generations of the above two species have been collected, they were found attached to the boulders of the Elphinstone

Bridge and of the small bridge near San Thome. During the January-March period of 1934 and 1935, fairly luxuriant growths of these Hydroids were observed and both the species showed gonosomes during the months January and February. The first species disappeared with the advent of summer, but colonies of *O. spinulosa* were found on dead oyster shells, stones, etc., even during the month of April, none of these however, showed gonosomes. The species was very rare in the month of June, but a month hence specimens could not be found. The next time they were observed again was in the succeeding season of high salinity after the bar had opened and the monsoon had ceased.

Our observations lead to the view that both the species have not permanently established in the backwater in the sense that the colonies do not survive and actively reproduce throughout the year. In all probability they are only casual migrants, entering the backwater every year when the bar is open and the colonies thus started (and which may attain sexual maturity) almost completely perish with the advent of summer. Both these Hydroids are known from the brackish-waters in other parts of India (*vide* Leloup, 1932). Luxuriant growths of these may be observed in the Madras Harbour.

CLASS . *Scyphozoa*

Acromitus flagellatus (Haeckel)

This is the only Scyphozoan that occurs in the Adyar, the Cooum and the Buckingham Canal. The species enjoys a wide distribution throughout the coasts of India and is found in the sea, brackish-water, and even in water that is of very low salinity (Rao, 1931). Rao considers that *A. rabanchatu* described by Annandale from the Chilka Lake may after all be only a variety of *A. flagellatus*. At Madras the jelly-fish is found in the backwater all through the year, and we have collected specimens measuring from 5 mm to 200 mm across the bell.

Ephyrae.—During the November-January periods of 1934-35 and 1935-36 a number of ephyrae were obtained in the tow-net collections taken from the river as well as the backwater. During these years the ephyrae were not collected in any other season. As they were capable of surviving considerable lowering of salinity and were common in the backwater during this season the ephyrae were considered as belonging to *Acromitus flagellatus*; but their exclusive occurrence during the period when the bar is open, led us to think that these were brought down from the sea. No evidence was available at that time to prove that the process of strobilisation takes place in the brackish-water, but definite data bearing on this point was obtained in 1936. In describing the fauna of isolated brackish-water pools in our

previous paper we have specially referred to a large salt pool situated near the Adyar Boat Club. During June and July 1936, adult jelly-fishes were completely absent from this pool, and the water was but a few inches deep. The rains during August increased the level of water and the plankton collections made in August and September brought in plenty of ephyrae from the pool that was not at all directly communicated to the Adyar river at that time, in a season when the bar was closed. There is not the least doubt therefore that asexual phase of reproduction takes place in the brackish-water. In spite of careful search we have not so far obtained the strobila. Young specimens of the jelly-fish were observed in the pool by about October and November. So far as we are aware there is no previous record of the ephyrae of this species.

The ephyrae are about 1 to 2 mm across, they are perfectly transparent and unpigmented. In essential features they are very similar to those of *Aurelia* usually figured in text-books. The sense organs at the points of bifurcation of the arms are quite distinct in live specimens. In none of them were the canal system and other structures developed.

Very young individuals of the jelly-fish have been collected during the months of November, December, January and February. These were immature and the terminal filaments of the arms were well developed, similar to the condition reported by Annandale (1915) in *Acromitus rabanchatu*. Large specimens of the jelly-fish are found all through the year, but they are commoner in March and April and later in August and September, than at other times of the year. Several specimens collected at this time had well-developed gonads with ripe eggs or sperms as the case may be. We have not seen them spawn, nor have we found planulae in the tow-net collections. Menon (1930) finds that they begin to appear in August till some time in November. His record of the largest specimen (which was immature) was in October. There is a certain amount of variation in the time of their appearance, but summing up the records, it seems probable that both the asexual and sexual phases of reproduction take place in the brackish-water, the asexual phase being produced during the months August-January, and the sexual phase probably taking place during the other half of the year. It must also be admitted that large numbers of jelly-fish enter the back-water from the sea when the bar is open.

The observations of Annandale are noteworthy in this connexion. In Chilka Lake, he says that probably the fixed stage of *A. rabanchatu* occurs on rocks or weeds near the south end of the Lake, where the young were found in April, July, September and November, but not in January or February. If this were so, it would mean that at Madras, the liberation of the

ephyrae takes place much later in the year than it does in the Chilka Lake Annandale also says that the main breeding season of *A. rabanchatu* as judged by the condition of gonads in the specimens occurs towards the end of the cold weather, i.e., February and March (p. 102)

CLASS *Anthozoa*
ORDER *Actiniaria*

Phytocætes gangeticus Annandale

This anemone breeds all through the year, young ones, post-larval stages, and sexually mature individuals have been observed during all months (*vide* Panikkar, 1937). In addition to the information given in the above-mentioned paper, an interesting point to be noted here is that concerning the proportion of sexes. In all collections made, the anemones were either exclusively females, or the majority of them were so, male anemones having been somewhat rare.

Phytocæteopsis ramunni Panikkar

Stephensonactis ornata Panikkar

Repeated examination of the gonads of both these species were made for about a year and a half from December 1933. The gonads were both examined in the fresh state and also from sections. Ripe ova and spermatozoa have been observed all through the year, both when the bar was open and when closed (Panikkar, 1936). Artificial fertilizations were not; however, successful, nor have we collected larval and post-larval stages.

Pelocætes exul Annandale

Ripe sperms and ova have been observed throughout the year. One of us has already pointed out that Annandale's view that the species is hermaphrodite is not supported by careful anatomical investigation (Panikkar, 1938).

Boloceractis gopalan Panikkar

The species reproduces both by asexual and sexual methods as has been described in a previous paper (Panikkar, 1937).

PHYLUM *Annelida*
CLASS *Polychæta*

Lycastis indica Southern

This polychæte is almost amphibious in habits, and it has been taken from localities where the water is almost fresh. One of us (Aiyar, 1935) has noted that the species is hermaphrodite and probably protandrous as judged by the size of the worms either preponderatingly male or female. Mature

specimens with fully developed eggs and sperms have been observed all through the year, the developmental stages have not so far been collected.

Diopatra variabilis Southern

The development of the eggs of this species takes place inside the tubes of the parent worm (Krishnan, 1936). Taking the records of the past three years, tubes with embryos of *Diopatra* have been taken from the Adyar backwaters during all the months of the year. This would mean that the species breeds all through the year, though we have no information whether there is an intensive spell during any particular period.

Marphysa gravelyi Southern

The worm breeds all through the year (Aiyar, 1931) and the characteristic spawn masses of this worm are extremely common all along the mud flats, shallow pools, banks of the Adyar river and backwater, Cooum and the Buckingham Canal throughout the year. The largest amount of spawn masses occurs from August–February, especially during the few weeks that follow any outbreak of monsoon. There is an appreciable decline in the number of the spawn during the period of drought (April–June).

Lumbriconereis sp

The species is different from all other members of the genus known from India and is being described in detail separately by one of us (R. G. A.). They are exceedingly common in the mud, especially in the surface zone. Worms having fully developed ova have been noted during all times of the year.

Scoloplos sp

The species has not so far been settled, but it is specifically distinct from *S. marsupialis* recorded from the Chilka Lake by Southern (1921) and *S. latus* and *S. kerguelensis* the two other species recorded from other parts of India by Fauvel (1932). The worm has been obtained all through the year. Like *Marphysa* the eggs are collected into gelatinous masses, though the spawn is much smaller being only 8 to 10 mm across. They are fairly common in the backwater during the period when the bar is closed, from August–October (1934, 1935), in 1936, they were also found between March and July. Details of habits and development of this Polychæte are dealt with in another paper (Aiyar, R. G., unpublished, 1938).

Hydroides norvegica Gunnerus

This species seems only to be a casual migrant to the river and backwater since live specimens were not observed in the brackish-water during all months of the year. The worms begin to appear along the boulders of

the Elphinstone Bridge attached to oyster shells and stones, about a month after the opening of the bar and they are observed here for a few of the succeeding months. By about May, only the empty calcareous tubes of the polychæte are observed. The species is abundant throughout the Madras coast and is very common in the Madras Harbour. Every year the larvæ are probably washed into the Adyar river and backwater when the bar is open, and they establish on suitable substrata, but a very high percentage of worms perish with the onset of low salinity conditions and drought. M D Paul (unpublished) has found out that in the Madras Harbour the worm is a continuous breeder and that sexual maturity is attained rapidly within a few weeks after the attachment of the larva.

Pontodrilus bermudensis Beddard

Specimens collected during December 1934, February 1934 and January 1935 had well-developed clitella. Unfortunately we have not collected worms during other months of the year and hence no definite information on breeding could be given here. However, the above records are in conformity with Stephenson's observations (1915) on the specimens of the Chilka Lake, that the breeding time appears to be late in winter and early in spring.

PHYLUM . . . *Arthropoda*
CLASS . . . *Crustacea*
ORDER . . . *Cirripedia*

Balanus amphitrite Darwin

As in the case of *Hydroides norvegica* the species is common only during the months when the bar remains open. They are found attached to stones, boulders of the two bridges, shells of hermit crabs, and oysters. From the plankton records given in our account of the Adyar fauna, it will be apparent that the larvæ are brought down from the sea when the bar is open and they settle down on suitable substrata. The individuals survive in the brackish-water for some months, and most of them perish with the advent of drought when the level of water falls down from the situations where they have settled. It is probable that they are able to reproduce in the brackish-water during the few months of their annual existence. In the Madras coast the species is a continuous breeder (Paul, 1938, unpublished).

ORDER . . . *Schizopoda*.

Mesopodopsis orientalis (Tattersal)

Females with embryos are observed during all months of the year in collections made from the Adyar backwaters and the Cooum, and the species is capable of reproducing in brackish-water as well as sea water. Details

of the development and life-history of this Mysid are given in another paper by Mr K B Nair (1939)

Rhopalophthalmus egregius Hansen

Swarms of this Mysid were found during November and December 1933, and collections included plenty of females with embryos of different stages in the brood pouches. The bar was open at this time and as the species was absent in subsequent plankton collections we were inclined to consider that the species is only a casual migrant from the sea. But there is not the least doubt that the species is capable of surviving and breeding in the brackish-water since we have obtained specimens on one occasion when the bar was closed (12th August 1935). A few males and females with embryos were obtained in the tow-net from the middle zone of the backwater. Though a number of plankton collections have been made within the course of our study, there has never been any further record of this Mysid from the brackish-water. The species is common in the Madras coast during February, March and April (*vide* Aiyar, Menon and Menon, 1936)

ORDER

Amphipoda

Grandidierella magna (Giles)

Grandidierella gilesi Chilton

There is considerable difficulty in determining the two species since systematists are not agreed on the synonymy of these and allied species, we have followed only the descriptions given by Chilton (1921). As judged by the females carrying embryos, both the species breed all through the year.

Paracalliope fluviatilis (Thomson)

Females with embryos have been noticed irrespective of seasons

ORDER

Isopoda

Cymothoa indica Schiodte and Meinert

Embryos of this parasitic Isopod have been taken from females during all months of the year (Panikkar and Aiyar, 1937a). In addition to the host-fishes recorded before by us, we wish to add the goby *Acentrogobius viridipunctatus* (Day) and *Macrones* spp in which also the parasites are occasionally found¹

Cyrolana sp

Eggs and embryos have been noted during November and December 1935. There are no records for other periods

¹ Kindly brought to our notice by Mr. T. J. Job.

Ligia exotica Roux

This is a completely terrestrial Isopod, but never occurs far away from the water edge. Young specimens as well as large females with eggs in brood pouches have been recorded during the months of July, September, October, December and January. There is a decrease in the number of individuals from February onwards, and during May they are apparently absent from their usual haunts on the boulders of the bridges, but may be observed between stones and crevices. An individual with eggs was once noted even during May. It is probable that the species breeds during the major portion of the year, though the summer months are presumably less favourable than winter and spring.

SUB-ORDER *Macrura*

ORDER *Decapoda*

Periclimenes indica (Kemp)

The species is a regular breeder in the backwaters since ovigerous females are commonly obtained during the months when the bar is closed, especially during April, June and July. In 1936, ovigerous females have also been obtained during September and October. In Chilka Lake ovigerous females are found in February, March, July, September, and Kemp (1915) says that the species breeds only in water containing some trace of salinity. From the backwaters of Ennur, the same author has recorded females with eggs in January (p. 229). From all these records it is not possible to say whether there is a well-marked breeding season for the species. The total absence of ovigerous females during the months when the bar is open at Adyar suggests that the species does not breed when the salinity is high.*

Alpheus malabaricus Fabricius

Though the species is a permanent inhabitant of the backwater and may be obtained all through the year, ovigerous females have been noted only during December and January. Young specimens are quite common in the backwater during January and February. As regards other alpheids, no ovigerous female has been collected by us from the brackish-water.

Penaeus carinatus Dana

Penaeus indicus Milne Edwards

Specimens of these two species of marine prawns which enjoy a wide distribution in the Indo-Pacific, may be collected from the brackish-water

* These observations are also true of certain other *Palaeomonidae*, the ovigerous females of which we have collected, but have not been able to identify.

at any time of the year As suggested by Annandale (1922) for the Gangetic delta, Kemp (1915) for the Chilka Lake, Dakin (1935) for the New South Wales coast and Panikkar (1937) for the Malabar coasts, these species do not breed in the brackish-water but only in the sea They enter the backwaters and lakes during their larval or post-larval stages and grow there for about an year, after which they go back to the sea to breed At Madras the matter has been investigated in detail by Mr M. A. John (1936,* unpublished) with reference to the conditions at Adyar and in the Cooum He finds that migration takes place when the prawns are half-an-inch to one inch long and grow in the backwaters to a maximum size of five inches, while the sexually mature adults from the sea are about 9 inches long Our plankton records for the Adyar backwaters (*vide* pp 311-13, Panikkar and Aiyar, 1937) show that the post-larval individuals enter the backwaters during all months when the bar is open

Penaeopsis monoceros (Fabricius)

Specimens of *Penaeopsis monoceros* are common in the backwater during all months of the year Young specimens 15 to 20 mm long have been obtained in tow-net collections taken in September and October when the bar is closed. Slightly older examples 20 to 30 mm long are also obtained in the ordinary nets Judged from their size, these individuals could not be more than one to two months old, while about six months had elapsed since the bar closed This would point to the conclusion that the species can breed in brackish-water unlike the other two species of Penaeids and breeding in this particular years (1935-36) must have taken place when the bar remained close It is hoped that further investigations will result in the larval and post-larval stages also being collected when the bar is closed It is noteworthy that in the Chilka Lake, Kemp (1915) thinks it improbable that any of the species of *Penaeopsis* breed there

SUB-ORDER . *Brachyura*

Matula victor Fabricius

Ovigerous females of this species have not so far been collected.

Ocypoda cordimana Desmarest

Ovigerous females have been collected during January and February from Adyar, and during December-March from the Cooum bar, but not in any other month of the year.

* " Migration of Penaeid Prawns of Madras."

Ocypoda macrocera Milne Edwards

Ovigerous females noticed in December, January and February. Megalopae belonging to this species and identical in appearance with the figures given by Kemp (1915) have been collected during January 1933 and 1934.

Uca (Gelasimus) annulipes (Latreille)

The species is extremely common both at Adyar backwaters and near the bar of the Cooum river near the Laboratory. Several specimens have been collected and observed during all months of the year, but ovigerous females have been found only during the months of October, November, December and January.

Metaplex distincia (Milne Edwards)

The species is not so common as the other crabs and we have collected specimens only from November and April. The only two ovigerous females so far obtained (both from an island in the Adyar river near the Elphinstone Bridge) were found in the month of January 1935.

Varuna literata (Fabricius)

Ovigerous females have not been collected by us; but the species is one that commonly breeds in the brackish-water even if the salinity be very low as evidence by Annandale's findings in the Gangetic delta (1922).

Sesarma tetragonum (Fabricius)

The species is common all through the year and the records of ovigerous females are for the months of October, November, December and January. During these months the crabs, especially the males, are beautifully coloured, the species is rarely seen out of the burrows in the summer months and the brilliant coloration is not noticed in specimens that are dug out.

Sesarma quadratum (Fabricius)

Ovigerous females are quite common during November, December and January. In 1937 the season extended even to February. The ripe females were absent from the collections made in the other seasons.

Scylla serrata (Forsk.)

Neptunus pelagicus (Linn.)

Neptunus sanguinolentus (Herbst)

None of these crabs is represented in our collections by ovigerous females. The last two species are no doubt casual migrants since specimens have been noted only when the bar was open, and that not far away from

the bar *Scylla serrata*, however, is a permanent inhabitant common all through the year from the bar to about three miles up the river. Specimens are also met with in the Cooum and the Buckingham Canal. Within the period of our study we have examined over 200 specimens but the collections do not contain a single ovigerous female. There is also a marked disproportion in the number of males and females since female individuals are encountered much less frequently than the males.

SUB-ORDER

*Anomura**Paguridea*.*Chibanarius olivaceus* Henderson*Chibanarius padavensis* De Mann

Both these species of hermit crabs are perennial breeders in the Madras brackish-waters. Subramaniam (1935) has noted that in *C. olivaceus* the reproductive activity is highest from September–March. He has also brought in evidence to show that the sizes of the eggs and their cytoplasmic components are different in the January–February and April–June periods. Fully mature sperms have been noted during all months of the year in *Chibanarius padavensis* (Ratnavathi, unpublished observations). Judged by our collections of berried females we are inclined to consider that though both the species are continuous breeders, the breeding is especially intense during the time when the bar remains open.

PHYLUM

*Mollusca**Lamellibranchiata**Ostrea arakanensis* Sowerby

The species has been known under various names as *O. virginica*, *O. madrasensis*, etc (vide Winckworth, 1931). At Adyar the oysters occur below the Elphinstone Bridge and in the embankments of the Theosophical Society's quarters, and occasionally near the small bridge. The only observation that we have to record is the occurrence of specimens with motile sperms during November 1934, and October and November 1935. Hornell (1922) mentions that in the Coromandel coast the chief spawning period is from August–September, with a secondary period during March and April. He also observed that under normal conditions the Indian Oyster is a most irregular spawner and that ripe individuals may always be found except when the floods are on.

Modiolus undulatus (Dunker)*Modiolus striatulus* Hanley

Specimens of both these bivalves with ripe sperms and ova have been noted during January, March, April, May, August and September. For other

months we do not unfortunately have any records. Numerous young individuals belonging to the first species were common during March-May in 1935

Meretrix casta Chemnitz

Specimens with ripe eggs and sperms are obtained during all months of the year (Rama Varma, unpublished observations). The species therefore probably breeds during all the seasons, but there is not the least doubt that the reproductive season is at its height during the early summer months. We have not noted actual spawning of the live clams, but large numbers of very young individuals are to be found during April, May and June. Hornell (1922) observes that spawning appears to take place twice a year, the first during April-May and then again about September. It is probable that these two periods noted by Hornell represent active phases of spawning in the clams with much less reproductive activity during the intervening period.

Gastropoda

Stenothyra blanfordiana Nevil

Amnicola (Alocinnia) stenothyroides (Dohrn)

Ripe individuals and very young ones of the above Gastropods are to be noticed at any time of the year, but here also there is a marked intensity of reproduction during the summer months, when the bar is closed. The intensity of reproduction in these two species is so high during this season that they constitute dense animal communities, numbering even up to about a hundred individuals per square inch.

Potamides cingulatus Gmelin

Individuals with ripe sperms have been noted during all months, but here active breeding does not take place all the year round. The species produces characteristic vermiform spawn masses consisting of mucus, mud and sand grains, in which are imbedded the eggs. Each egg is closely agglutinated to a sand grain. The spawns left by the animals are quite common in the mud and shallow pools during the summer months of the year, as many as 20 spawn threads per square foot have been collected on an average during the months March, April and May 1936. The spawn begins to appear on the mud flats during January and the number increases with the gradual onset of summer, the maximum observed being in April-July. Their numbers fall very considerably after July and they are almost completely absent in the November-January period.

Stiliger gopalai Rao

Rao (1937) reports that mature individuals of this Nudibranch (*AscoGLOSSA*) are obtained at all times of the year and that therefore no definite

breeding period is clear, but since low salinity conditions have been found to be essential for metamorphosis, the breeding is to a great extent determined by the periods of rainfall. The species is frequently noted during the warmer months of the year, crawling amidst the alga *Chatomorpha* which abounds in this season. The animals are not so common during other periods and are not observed at Adyar when the bar is open. The development and metamorphosis are accomplished within a short period and several generations of actively reproducing specimens may be observed in any favourable season.

Unidentified Spawn Masses

Spawn masses belonging to other species of Gastropods have been found from February–June during all the three years of our study. We have not been able to refer them to their adult species. The egg ribbons of *Natica* sp. similar to the figure given by Hornell (1922) have been observed at Adyar in March 1934, March and April 1935, and April 1936, and near the Cooum bar in March 1936. They are found along with the spawn of *Potamides cingulatus*. Adults of *Natica* have not so far been collected by us either from the Adyar or from the Cooum.

VERTEBRATA .

*Teleostei*⁴

Notes on the breeding habits of the brackish-water fishes of Madras are contained in the papers of Raj (1916), Aiyar (1935) and Jones (1937). In the following account we give a summary of observations contained in those papers along with the notes that we have been able to gather during our period of study.

SUB-ORDER . *Malacopterygi*

Larval and post-larval stages belonging to the genera *Engraulis* *stolephorus* and *Elops* have been collected in the tow-nets during the months of November, December and January. These belong mostly to the groups of species usually noticed in the local marine plankton and are mostly forms entering the backwater with the opening of the bar. Our records are insufficient to conclusively say that any species of the Malacopterygi breeds in the brackish-water. However, Raj (1916) says that in Madras, *Elops indicus* is an essentially estuarine fish that easily acclimatizes to fresh-water and that young fish are quite common in the Cooum, the breeding season being the cold weather. As regards *Megalops cyprinoides* he reports that larvae are common in the Cooum in October and November in fresh-water. Among

⁴ The following species may be added to the list of fishes given in our paper on the Adyar fauna.

the Clupeidæ, he finds the larvæ of *Engraulis purava* in the Cooum in October and November, which according to him is the breeding season

SUB-ORDER .. *Ostariophysæ*

The breeding season of *Barbus sophore* is mentioned by Raj to be the cold weather, i.e., from November-January. We have collected numerous young ones of this fish during this season. Among the Siluridæ, *Arilus falcatus* Richardson is mentioned by Raj as a common estuarine species and that one of the breeding grounds of the fish in Madras is the mouth of the Adyar river, the season extending from September to November. He also records ripe females with eggs for the species *Macrones villatus*

SUB-ORDER . *Apodes*

Anguilla elphinstonei is recorded by Raj, who found large numbers of eelers measuring 2 or 3 inches in length ascending the Adyar and Cooum in November. The species is not represented in our collections

SUB-ORDER .. *Haplomi*

The two common Cyprinodont fishes of the Madras brackish-waters are *Aplocheilichthys melastigma* and *Panchax parvus*. Both the species are perennial spawners but the intensive period of reproductive activity for the former species is from September to February, and for the latter from January to February (Raj, 1916, Jones, 1937)

SUB-ORDER *Percesoces*

Jones has recorded the larvæ of *Hemirhamphus gaimardi*⁵ during the month of November and the eggs of *Tylosurus strongylurus* were obtained by Job and Jones in October 1937⁶. They also record gravid females for the month of December

So far as the Mugils are concerned the active breeding period is mainly the cold weather as evidenced by our collection of large numbers of young ones soon after the monsoon

SUB-ORDER *Acanthopterygiæ*

Among the Serranidæ, young ones of different species enter the backwaters in their young stages and after they have grown for some time, they go back to the sea. Rao (1934) found that in *Therapon jarbua* the first yearlings are found in the Adyar backwater while sexually mature ones are

⁵ Syn. *H. limbatus* (vide Weber and Beaufort's *Fishes of the Indo-Australian Archipelago*).

⁶ "Studies on the Development of the Indian Gar-fish *Tylosurus strongylurus* (Van Hass.) with Notes on the Young Stages of *Hemirhamphus gaimardi* O and V., " *Bull. Ind. Mus.*, Vol. XL, Pt. III.

only found in the sea. *Ambassis mops* is a species that breeds in the brackish-water, it is common in the Adyar and the Cooum in November and December, which is the breeding season according to Raj (1916). We have obtained sexually mature individuals in January and February as well. The reproductive habits of the Adyar Gobies are reported by Raj (1916), Aiyar (1935) and Jones (1937). *Acentrogobius neilli* and *Acentrogobius viridipunctatus* are typical perennial spawners, but reproduction is especially active when there are rains, i.e., during September–November. The latter period may be considered as the breeding season for *Glossogobius aureus* and *Eleotris fusca* as judged by our records as well as Raj's statement. In regard to *A. aureus*, Hora (1936) mentions that it is one of the few gobies that migrate to the sea for breeding purposes. It is probable that the Adyar and Cooum river mouths form the spawning grounds of this species which is common in the freshwater localities of Madras.

SUB-ORDER *Zeorhombi*

Among the Blennies, Jones records the eggs of *Petroscirtes bhattacharya* in January 1936. In the Cichlid fish *Etroplus maculatus*, Raj finds that the spawning season extends from January–April, most fish breeding between February and March. Jones observed the eggs from August 1935 onwards. He also reports that this species (as well as *E. suratensis*) goes up the river into less saline regions when the bar remains open and that it comes back only when the variations in salinity are not sudden. With regard to *E. suratensis* he says that the eggs are not laid when the sea water comes in, developmental stages having not been met with when the bar remains open. Hence both the species are forms that can breed only under low salinity conditions.

Summarising the observations on breeding in fishes inhabiting the Adyar and the Cooum, it may be mentioned that a good many species breed in the cold weather between September and January. In most cases the breeding is especially active during the monsoon. Even perennial spawners like *Acentrogobius neilli*, *Acentrogobius viridipunctatus*, *Aplocheilichthys melastigma* and *Panchax parvus* exhibit intense reproductive activity during the rainy weather. Further, outbreaks of rain during the summer have been observed to stimulate the reproductive activity of these species as evidenced by the large number of fish fry that may be observed a few days after such showers. Even in the continuous breeders of this type, we have reason to think that egg-laying by the ripe females is very considerably augmented by the rains so much so that intensive spells of spawning might be occasioned by outbreaks of rain, the changes in the environment that they cause probably acting as a definite stimulus.

General Considerations

The study of breeding in marine organisms has come to the fore at the present day and a good amount of field and experimental work is now carried out in different parts of the world on breeding and propagation of marine invertebrates. One of the problems confronting the marine zoologist is the difference in the reproductive behaviour of animals inhabiting different latitudes. In his account of the natural conditions as they affect animal life, Semper (1885) emphasized the difference in reproductive habits of animals living under the tropical and temperate conditions. The effect of tropical climates, where variations in temperature are reduced to a minimum, is, according to him, the obliteration of the periodicity of animal life which is so striking in climates that vary between two remote extremes of winter and summer. The breeding habits of the animals of the North Sea and English Channel have been studied by a number of investigators and the restricted breeding seasons for the majority of species inhabiting these localities is now a well-established fact. Even in the Mediterranean which is warmer than other temperate seas, the extensive researches of Lo Bianco (1909) at Naples showed that the breeding of most animals is confined to certain definite periods of the year varying according to the species concerned. It may be noted that the actual spawning periods of several species are longer in the Mediterranean than the corresponding periods for similar or even identical species found in the Channel or in the North Sea. In many Invertebrates, the periodicity is often eliminated even in colder seas (Semper). Semper states "Nothing in the Philippine Islands struck me so much as to observe that there all true periodicity has disappeared even from insects, land molluscs, and other land animals, I could at all times find eggs, larvæ and propagating individuals in winter as well as in summer. It is true that drought occasions a certain periodicity, which is chiefly perceptible by the reduced number of individuals in the dry months and the greater number in the wet ones; it would seem that a much smaller number of eggs are hatched under great drought than when the air is very moist" (p 135). "But what was far more striking in these islands was the total absence of all periodicity in the life of the sea animals, particularly the invertebrata; among these I could not detect a single species of which I could not at all seasons find fully grown specimens, young ones and freshly deposited eggs" (p 136).

The publication of Orton's paper (1920) on sea temperature and breeding in marine animals gave a fresh impetus to the subject. Giving a clear analysis of previous literature and his own observations on the breeding of several species, he emphasized the paramount importance of sea temperature in controlling breeding in marine animals. In an earlier contribution,

Appelhof (1912) indicated the importance of suitable temperatures for the reproduction of several species of marine animals and also discussed the rôle of temperature in the distribution of the species. Though Orton's conclusions were rather anticipated by Appelhof, he did not attempt at a definite correlation between the two. Orton arrived at the general conclusion that, in general, a species breeds for a longer period in the more warmer southern latitudes of its distribution, since the temperature in such situations will remain for a longer period above the lowest temperature at which the particular species can breed. In a series of recent contributions, Runnström (1927-29) has experimentally proved this in regard to the breeding habits of a number of Arctic, Boreal and Mediterranean species.

While we do not wish to enter into a discussion of the topic in this paper a few generalizations in this connexion appear to us as being likely to facilitate a clearer grasp of the problem. This is especially necessary because the observations of Semper (1885) and Orton (1920) have often led to a belief, which is widely prevalent, that all tropical marine animals breed continuously irrespective of the seasons. In a recent paper, Galtsoff (1935) has rightly criticized the indiscriminate remark found in text-books that under stenothermal conditions all marine animals breed continuously. He even asserts that in the tropics as well as in the temperate regions there is no animal that actually breeds continuously, but that always we meet with only periodic breeding. We agree with Galtsoff in affirming that the usual belief in continuous breeding of all tropical marine species is erroneous; but we are unable to accept the view that periodicity is universally observed. First of all it is not clear whether in mentioning the occurrence of periodicity, he really refers to animals considered as individuals or as communities. The development of sexual products in any organism is a periodic phenomenon inasmuch as it occurs during some definite phase which may happen only once or may be rhythmically repeated, and not throughout the life-history of the individual. However, this need not bring about periodicity in breeding. In several species, the individual reproductive rhythms do not coincide and we may find at the same time specimens with ripe eggs and spent gonads as well as those showing the intermediate stages. The periodicity in these individuals is purely individual and not racial, consequently the process of spawning, development and attainment of sexual maturity occur simultaneously if we consider the individuals of any particular species collectively as a single community. The breeding in such animals may be said to be continuous. In the other set of animals the individual reproductive phases of the organisms constituting a community closely approximate so that spawning, development, and attainment of sexual maturity occur in certain well

marked periods, the community may be said to develop, grow and spawn together. In all such animals a definite periodicity will be observed. But this periodicity tends to become obliterated when the reproductive phases appear in quick succession and several generations of the animals are involved in breeding, such species would appear again as almost continuous spawners. It may be emphasized here that both continuous as well as periodic breeders in the sense we have explained above may be met with in nature. The study of breeding in marine invertebrates of the Great Barrier has brought to light the existence of different types of breeding in this tropical habitat—continuous, discontinuous and even discontinuous breeding in relation to lunar phases (Stephenson, 1935). Recent work conducted in this Laboratory by Mr M D Paul has amply borne out this point, for, among the organisms of the Madras Harbour, he has been able to observe continuous and discontinuous breeding among certain sedentary organisms, while we have described discontinuous breeding in relation to lunar phases in a Polychaete from the same environs (Aiyar and Panikkar, 1937). We also wish to mention that to a considerable extent the periodicity in reproduction has something to do with the span of life of the individuals constituting the species. From his study of the rate of growth, age at sexual maturity and duration of life in sessile organisms at Woods Hole, Grave (1933) concludes that some of the most prolific animals were those that live for less than one year. In such organisms several generations are produced in quick succession and the intense reproductive activity observed in them does not reveal regular periodicity.

Summary and Conclusions

1 Breeding of animals of the brackish-waters of Madras is not particularly confined to any definite part of the year, actively reproducing species, especially the invertebrates, are met with during all seasons. In a good many forms there is intense reproductive activity during the rainy weather.

2. The following types of breeding were noted —

- (a) Continuous breeding throughout the year occurring more or less uniformly and irrespective of seasons
- (b) Continuous breeding with marked season of higher activity in part of the year than in the remaining period
- (c) Breeding period confined to some definite part of the year
- (d) Discontinuous breeding occurring all through the year, breeding often taking place irregularly, mostly determined by the rains

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THE INTERNAL EAR OF *SCOLIODON* *SORRAKOWAH* (CUVIER)*

BY SHYAM SUNDER LAL

(From the Department of Zoology, St John's College, Agra)

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Introduction

WHILE conducting the B.Sc. practical class last year, Mr. Beni Charan Mahendra noted certain differences between the actual membranous labyrinth of the Indian shark (*Scoliodon*) and the figure¹ and description of that organ given by Thillayampalam in her Memoir (1928, rev. ed. 1938). As the Indian shark is now very commonly used all over India as a type of *Pisces*, it is obviously necessary to have as accurate and detailed a knowledge of its internal anatomy as possible. At Mr. Mahendra's suggestion, therefore, I have studied its internal ear carefully and I hope that the present paper, besides giving a correct description of this structure, may add something new to our knowledge of this organ in Elasmobranchs. I am grateful to Mr. Mahendra for his continuous guidance and assistance in the work, to Dr. Bains Prashad for the determination of the species, and to Prof. H. M. de Burlet (Groningen) for his helpful suggestions in connection with the Bibliography and for the present of many important papers for reference.

Historical Résumé

If we leave aside the numerous researches on the Internal Ear of Vertebrates in general and Fishes in particular and confine our attention only to the morphology of this organ in Elasmobranchs, Weber (1820) perhaps may be regarded as the first anatomist who dealt with it. He gave a general account of it in *Raja torpedo* and *Squalus carcharias*. In 1838, Breschet described the Internal Ear of *Squalus galeus*, *Chimæra callorhynchus* and the ray-fish. Hasse (1873) summed up the previous work on this organ in vertebrates, added much to our knowledge of it in several groups, and gave a historical résumé, including also an account of the work done on the Plagiostome Ear. Retzius (1872-1884), in the course of a series of admirable studies, made valuable contributions to the subject and described in detail the Internal Ears of *Acanthias vulgaris* and *Raja clavata*. Hellmann (1898) studied the development of the labyrinth in *Torpedo ocellata*, while Quiring

* Thesis approved as part of the requirements for the M.Sc. degree (1939) in the University of Agra.

¹ In fairness to Dr. Thillayampalam, it must be mentioned that the illustrations for the memoir are the work of Mr. M. Y. Mangrulkar and Dr. M. L. Bhatia.—B.O.M.

(1930) described the development of this organ in *Acanthias vulgaris*. Thillayampalam (1928, 1938)¹ has figured and concisely described the Internal Ear in *Scoliodon*, and de Burlet (1934) has given the best up-to-date account of the inner and middle ears in vertebrates

General Account of the Internal Ear

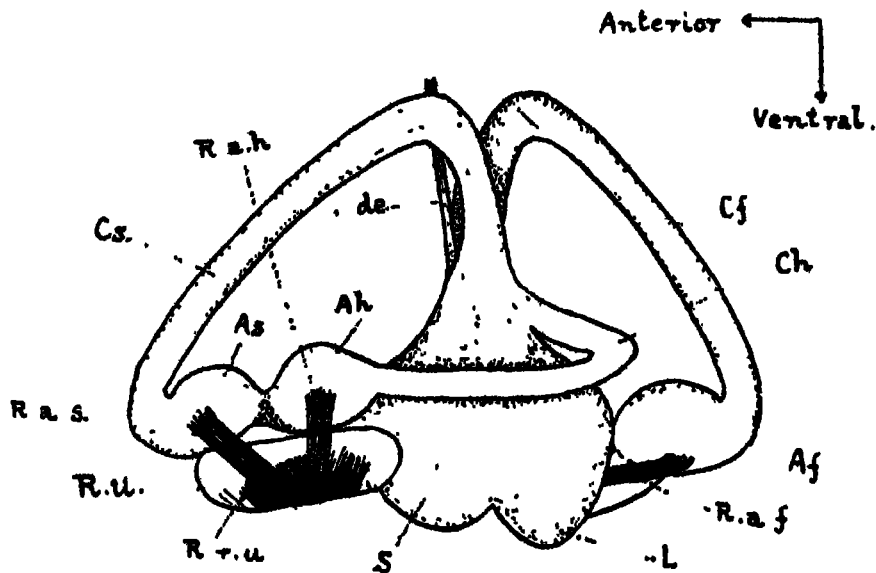
The Internal Ear or Membranous Labyrinth of *Scoliodon*, like that of other vertebrates, is a complicated, hollow organ full of endolymph and surrounded by perilymph. It is situated in a cartilaginous auditory capsule, on each side of the hind region of the cranium, and communicates to the outside by a delicate tube, the *ductus endolymphaticus*. It is supplied by the eighth auditory nerve and is divisible into a central part known as *otosaccus*, and three *semicircular canals* opening into this central part. One of these semicircular canals (*canalis horizontalis*) lies in a horizontal plane on the outer side of the otosaccus, while the other two are placed in a more or less vertical direction, one (*canalis sagittalis* or *anterior vertical semicircular canal*) at the anterior side and the other (*canalis frontalis* or *posterior vertical semicircular canal*) at the posterior. Each semicircular canal is dilated, at one end, to form an oval *ampulla* before opening into the otosaccus, the other end having no such dilatation. The *otosaccus* is divisible into two parts, a *utricle* with its *Sinus utriculi superior* and *Recessus utriculi*, and a *Sacculus* with which is attached a *Lagena cochleæ*. The *Sacculus* is produced above into a delicate tube, the *ductus endolymphaticus* or *Aquæductus vestibuli*.

An examination of the internal ear *in situ* shows several important features about its orientation in relation to the body of the shark. Its lowest part, the *lagena*, which is situated at a slightly more ventral plane than the rest of the ventral region of the otosaccus, lies almost flush with the palate. Its dorsalmost part, which is formed by the upper sweep of the vertical semicircular canals, is situated *only a very little* below the roof of the cranium. The total height of the organ from the top of the vertical semicircular canals to the lowest aspect of the *lagena* is 1 cm, or a little more, in a specimen 42–46 cm long from snout to vent, and this is only .2 cm less than the total height of the cranium. The horizontal semicircular canal lies almost parallel to the long axis of the cranium. The anterior semicircular canal sweeps, from its dorsalmost region, downwards, outwards and forwards in an oblique direction. The posterior semicircular canal is directed from above downwards in a direction opposite to that of the anterior canal; it runs downwards, outwards and backwards. The two vertical canals make an angle of 110° between them.

¹ The revised edition (1938) of Thillayampalam's *Memoir on Scoliodon* shows hardly any difference, as far as the Internal Ear is concerned, from the first edition published in 1928.

The Utriculus

The *Utriculus* of the membranous labyrinth of *Scoliodon* (Text-Figs 1, 3) is a wide, laterally compressed tubular portion extending backwards and



TEXT-FIG. 1.—The left membranous labyrinth of *Scoliodon sorrakowah* from the outer aspect ($\times 6$).

Af—ampulla frontalis; *Ah*—ampulla horizontalis, *As*—ampulla sagittalis, *Cf*—canalis frontalis; *Ch*—canalis horizontalis, *Cs*—canalis sagittalis, *de*, ductus endolymphaticus, *L*, lagena; *R.a.f*.—Ramulus ampullae frontalis; *R.a.h*.—Ramulus ampullae horizontalis; *R.a.s*.—Ramulus ampullae sagittalis, *R.r.u*.—Ramulus recessus utriculi; *R.u*.—Recessus utriculi; *S*—Sacculus

upwards from the place where the ampulla of the anterior semicircular canal opens. It can be divided into three regions: a dorsal region known as the *sinus utriculi superior*, a middle region forming the middle part of the utriculus and a ventrally-placed, pouch-like region, the *recessus utriculi*.

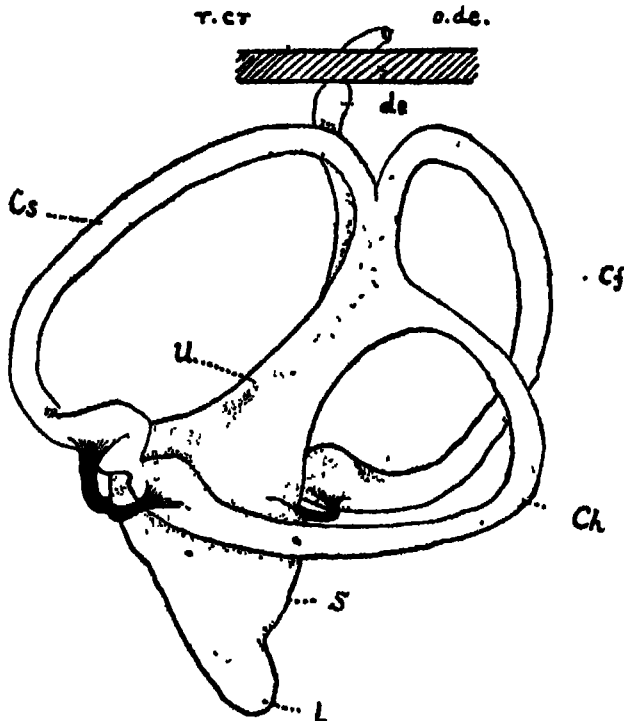
The *sinus utriculi superior* is the part of utriculus receiving the openings of those ends of the anterior and horizontal semicircular canals which do not bear ampullae. It is not sharply marked off from the middle region of the utriculus and is the name given by Retzius to the part situated posterior and dorsal to the *ductus utriculi* (*vide infra*).

The *middle region* of the utriculus receives the openings of the ampullated ends of both the anterior and the horizontal semicircular canals. The opening of the anterior semicircular canal is as wide as the part of the utriculus into which it opens, and is in a straight line with it. This means that the utriculus here appears as a direct continuation of the opening tube of the

sagittal ampulla (i.e., the ampulla of the anterior semicircular canal) The opening tube of the horizontal ampulla leads into the utriculus at the outer side, almost midway between the opening of the anterior semicircular canal and the ductus utriculi, it runs inwards at right angles to this region of the utriculus

The *recessus utriculi* is an antero-posteriorly elongated, laterally compressed, pouch-like region, lying adpressed below the two ampullæ and opening into the middle region of the utriculus. It opens by a wide aperture (*the ductus utriculi*) into the main part of the utriculus, and is superficially divided by a vertical groove into an anterior and a posterior part. The groove is only an apparent structure and serves for the passing along of the nerve branch to the two ampullæ

Thillayampalam (1928) has not only omitted the description of the various parts of the utriculus, but her figure of this organ (Text-Fig 2) shows definitely



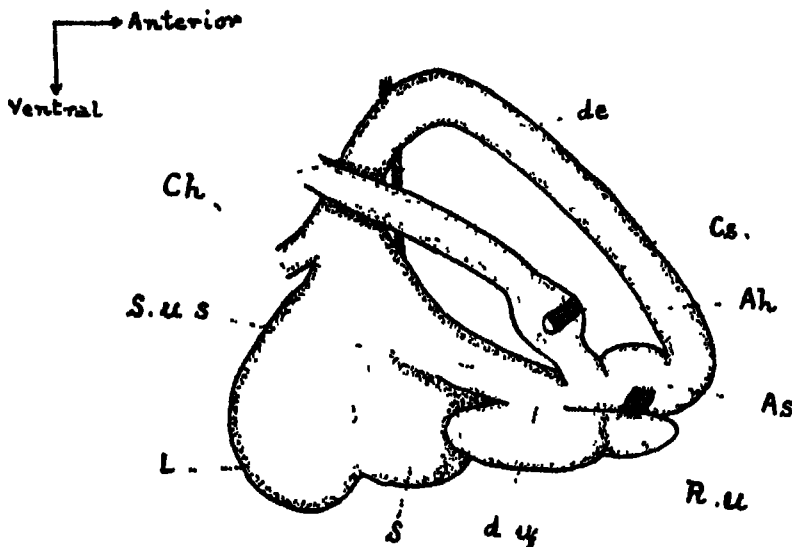
TEXT-FIG. 2.—The left membranous labyrinth of *Scyliodon*, as figured by Thillayampalam (1928). Amongst the main inaccuracies, note the joining of the two vertical canals dorsally to form a single tube, the position of the lagena ventral to the *Sacculus*, the absence of the *recessus utriculi*, the position of the horizontal ampulla lower to the anterior ampulla, and the dilatation of the ductus endolymphaticus.

o.de.—external opening of the *ductus endolymphaticus*; *r.cr.*—roof of the cranium; *U*—utriculus. The other letters as in Text-Fig. 1.

that she has completely missed the *recessus utriculi* and its opening into the rest of the utriculus. She portrays the horizontal ampulla as situated ventral to the ampulla belonging to the anterior semicircular canal, an observation clearly erroneous, due probably to her not having observed the internal ear *in situ*.

The Sacculus and the Lagena

The *sacculus* (Text-Fig 3) is a wide sac-like structure, laterally compressed and roundish in appearance, lying between the *recessus utriculi* on the one hand and the *lagena* on the other. It is produced at its dorsal end into a



TEXT-FIG 3 —Anterior part of the right membranous labyrinth of *Scoliodon sorrakowah* (outer view). The nerve branch of the *ampulla horizontalis* has been cut across and the horizontal semicircular canal is lifted up to show the utriculus and its parts ($\times 6$)

d.u.—ductus utriculi, *S.u.s.*—sinus utriculi superior. The other letters as in previous figures

delicate tube, the *ductus endolymphaticus*, which goes upwards to open on the roof of the cranium by a minute aperture. Posteriorly, the sacculus is in wide communication with a cup-shaped dilatation, the *lagena cochleæ*. Dorsally, it communicates with the posterior semicircular canal. I do not find a *ductus canalis frontalis* in *Scoliodon*, corresponding to what Retzius (1878) has described for *Raja clavata*. The posterior semicircular canal, on the other hand, opens directly into the sacculus by a dorso-ventrally elongated opening. Thillayampalam (1928) is wrong in her statement that each semicircular canal opens at both ends into the utriculus. The posterior semicircular canal, for example, opens not into the utriculus, but into the sacculus,

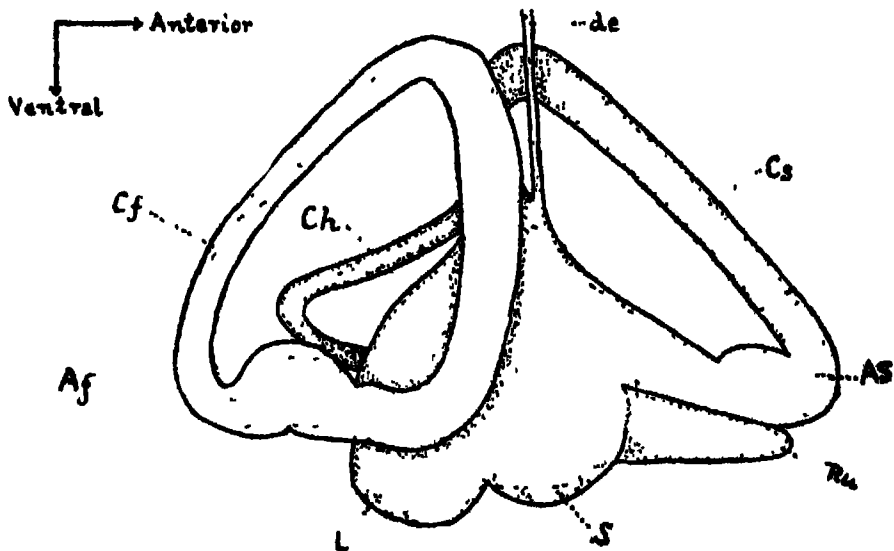
and has only one aperture into the otosaccus. As already known, this canal forms a closed circle with only a single point of communication with the rest of the membranous labyrinth.

The *ductus endolymphaticus* runs upwards, pierces the dorsal wall of the cranium at the region of the parietal fossa, and opens to the outside by a minute aperture on the skin just anterior to the commissural or supratemporal canal connecting the two lateral-line canals. It is a delicate tube uniform in width in its extent dorsal to the labyrinth and devoid of a dilatation as shown by Thillayampalam.

The Semi-circular Canals

The *anterior semicircular canal* or *canalis sagittalis* (Text-Figs 1 and 3) is broadly curved in its portion distant from the otosaccus, but bends rather angularly downwards at its dorsal end to lie outside the corresponding part of the posterior semicircular canal. It is of uniform width throughout its greater extent, but undergoes a gradual, slight dilatation before opening into the dorsal part of the *sinus utriculi superior*. Its ventral end also is angularly bent to pass into an oval dilatation, the *sagittal ampulla*, leading by a short tube inwards to the utriculus, and lying anterior to the ampulla of the horizontal canal.

The *posterior vertical semicircular canal* or *canalis frontalis* (Text-Figs 1 and 4) is divisible into three parts: a posterior or distal half, an anterior or



TEXT-FIG. 4.—The left membranous labyrinth of *Scoliodon varrakowah*, seen from the inner side. ($\times 8$). Lettering as in previous figures.

proximal half, and an *ampulla frontalis* separating the two halves ventrally. The posterior half is almost of the same diameter as the major part of the anterior semicircular canal, the anterior or proximal half is a little wider than it, and is the portion communicating with the sacculus. The whole posterior semicircular canal resembles a closed circle, pressed antero-posteriorly to approximate more or less to a triangle in appearance. Its proximal half lies mesially to the corresponding region of the anterior semicircular canal. Its ampulla is not quite oval in shape, but bears a concavity on its ventral surface.

Thillayampalam (1928) figures the two vertical semicircular canals as meeting together dorsally to form a common tube. This is incorrect. Seen from the outer aspect, the anterior semicircular canal lies over the corresponding part of the posterior semicircular canal. There is, however, no communication between these canals at all. As a matter of fact, the meeting together of the dorsal parts of these canals to form what is known as the *crus commune* is a condition found only in higher forms and could hardly have been expected in *Scoliodon* (cf. de Burlet, 1934).

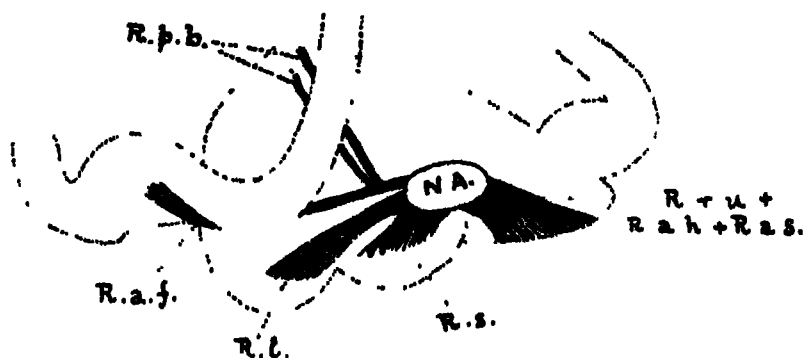
The *horizontal semicircular canal* is of a slightly less diameter than the other two canals, bears an ampulla (the *ampulla horizontalis*) at its anterior end, and opens at both ends into the utriculus. Its ampulla (Text-Fig. 3) leads into a short tube, which opens into the middle part of the utriculus just posterior to the opening of the ampulla sagittalis.

With reference to the openings of the semicircular canals into the otosacculus, Thillayampalam says that each canal opens at both ends into the utriculus. This statement, while applicable to the anterior and horizontal canals, is erroneous in the case of the posterior canal. As already mentioned the posterior semicircular canal opens not into the utriculus, but into the sacculus.

The Nerve Supply

As Thillayampalam's account of the innervation of the membranous labyrinth is both sketchy and inadequate, I give a more complete account of it below.

The membranous labyrinth (Text-Figs. 1, 5) is supplied by the eighth or *auditory nerve* (*Nervus acusticus*), which enters the cartilaginous auditory capsule a little above the boundary line separating the *recessus utriculus* from the *sacculus*. On reaching the labyrinth, this nerve immediately divides into two sets of branches: an anterior one (*Ramus vestibularis* or *R. anterior*), supplying the *recessus utriculi*, the *ampulla horizontalis* and the *ampulla*



TEXT-FIG. 5 —Inner view of the left membranous labyrinth of *Scoliodon sorrahensis*, to show the nerve supply

R l.—Ramulus lagenæ; *R p b*—Ranulus partis basilaris; *R s*—Ramulus sacculi. The other lettering as in Text-Fig. 1

sagittalis, and a posterior (*Ramus cochlearis* or *R posterior*), supplying the sacculus, lagena, ampulla frontalis and the dorsal part of the saccular wall

The *Ramus anterior* is divisible into three branches

(i) *Ramulus recessus utriculi*, consisting of numerous short fibres spread out more or less fan-wise over the ventral side of the recessus utriculi. They supply the *macula recessus utriculi*, the sensory end-spot in the wall of the utriculus

(ii) *Ramulus ampullæ sagittalis*, supplying the *crista acustica* of the sagittal ampulla. This Ramulus is spread out on the ventral region of the sagittal ampulla towards the outer side

(iii) *Ramulus ampullæ horizontalis*, supplying the *crista acustica* of the horizontal ampulla and spread on its outer side

Of these three branches, the *Ramulus recessus utriculi* was entirely missed by Thillayampalam

The *Ramus posterior* is also divisible into three branches

(i) *Ramulus sacculi*, supplying the *macula acustica sacculi* inside the sacculus. This nerve branch is distributed only on the inner side of the sacculus and is not visible in an outer view

(ii) *Ramulus lagenæ*, supplying the *macula acustica lagenæ* inside the lagena cochleæ. This branch also, like the previous one, is distributed on the inner side, being not visible in an outer view

(iii) *Ramulus ampullæ frontalis*, or the branch supplying the frontal ampulla. Shortly after its origin, it gives out two branches, running upwards on the wall of the sacculus, crossing the sagittal canal obliquely and

supplying the *Papilla neglecta* situated in this part Retzius (1878) regards these branches as corresponding to *Ramuli partis basilaris cochleæ* of bony fishes. The *Ramulus ampullæ frontalis* runs at first between the lower anterior end of the posterior semicircular canal and the lagena, and then comes to lie towards the inside of the ampulla frontalis, thus encircling its ventral aspect. It supplies the *cirista acustica* inside the frontal ampulla.

The *Ramuli partis basilaris* were not observed by Thillayampalam at all.

Summary

The author gives a careful and detailed account of the membranous labyrinth of the Indian shark *Scoliodon sorrakowah*, and both corrects and supplements the description of this organ given by Thillayampalam. Amongst the main inaccuracies in Thillayampalam's account, may be mentioned the joining of the posterior and anterior semicircular canals dorsally to form a single tube, the position of the lagena distinctly ventral to the sacculus, the entire omission of the *recessus utriculi* and its nerve supply, the missing of the two *Ramuli partis basilaris*, and the notion that the posterior semicircular canal also, like the other two canals, communicates with the utriculus.

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